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The Role of Substrate, Flow and Larval Supply to Recruitment of the Red Abalone (*Haliotis rufescens*)

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by

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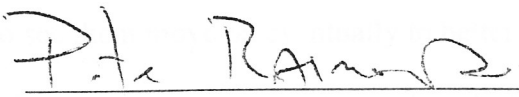
The thesis of Jessica Marianne Altstatt is approved.



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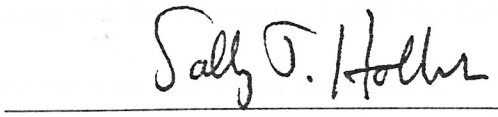

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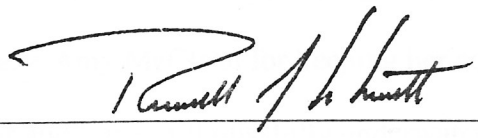

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ABSTRACT

Precipitous declines in wild populations of the red abalone *Haliotis rufescens* and the eventual closure of the commercial and southern recreational fishery have led to renewed interest in supplementing wild stocks with hatchery-raised individuals. Most work to date has focused on releasing small juveniles and has had limited success. Although much is known about larval settlement, juvenile survivorship and growth of abalone, there is scanty information on natural processes in the field. The failure of many regulated fisheries worldwide suggests that both the larval and juvenile stages may be important in determining the future population, and that early juvenile mortality is more important than previously believed. This paper presents a series of experiments designed to examine factors and mechanisms that could affect settlement, survivorship, and growth of larvae and early post-settlers in the field.

Laboratory trials under different flow regimes showed that red abalone larvae settled preferentially on substrates encrusted with coralline algae, and that settlement was rapid when exposed to crusts compared to other surfaces. Urchin grazing of films appeared to facilitate abalone settlement but only when urchins were removed. Initial field experiments showed that released larvae settled on natural cobble rock, and that settlement was at least one order of magnitude greater when settlement habitats were tented. I then examined post-settlement survivorship at one and two days after settlement, and found that although there was a large amount of variation, on average 10 % of released larvae were found as newly-settled recruits after 1 day.

Survivorship and growth of recruits were followed over at least one month in both Spring and Fall. Abalone settled at higher densities, survived better and grew faster in the warmer Fall months than in the Spring. The density of month-old abalone recruits was correlated with density of naturally-occurring gastropods in the Spring, but not in the Fall. These results suggest that settlement and survivorship can be extremely variable across space and time, and that oceanographic and local biotic conditions play a role and should be considered when planning larval seeding.

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Chapter One. Introduction to the Problem and Historical Background

For many benthic marine invertebrates, abundance of populations may be controlled by patterns of adult reproduction, larval availability, settlement and eventual recruitment into the adult population (Thorson 1950). Many of these processes are difficult to observe or to quantify, even if events such as spawning are well documented (Sewell and Watson 1993). Some species, such as rockfish (Love and Westphal 1981) or lobsters (Pringle 1986), have larvae that may remain in the plankton for up to a year before settlement, and thus may be carried hundreds or even thousands of kilometers from the natal reef. Other species, such as tunicates and bryozoans, may have very limited dispersal with larvae that settle within a few hours. Additional confounding factors may include the density of the spawning adults (Clavier 1992), the distance between the parental population and the settlement grounds (Palmer and Strathman 1981), the timing of localized current patterns which may affect larval transport (Shepherd *et al*. 1992, Stoner *et al*. 1997) or vertical distribution in the water column (Miron *et al*. 1995), and the availability of chemical cues (Morse and Morse 1984, Boettcher and Targett 1996) or favorable settlement substrate (Rowley 1989).

The relative importance of many of the factors listed above may be obscured by time lags between the actual settlement event and the time of sampling. Counting the numbers of individuals at some time period after initial settlement occurs may be misleading, as there is increasing evidence that high mortality may occur within a

short period after settlement (Keough and Downes 1982, McShane and Naylor 1995). Timing of settlement can often be back-calculated from sizes of post-settlers; however, many researchers feel that their sampling occurred close enough to the actual settlement event so as to be a reasonable measurement. In the literature, initial settler densities are estimated for various invertebrates as calculated from post-larvae sampled anywhere from 4-8 days (Luckenbach 1984), 5-17 days (Rowley 1989), 7-30 days (Pennings 1991) to 30-41 days (Harrold *et al.* 1991) after settlement.

Are these estimates good enough? Supply-side ecology suggests a strong and direct link between available larvae and eventual recruitment (Lewin 1986, Underwood and Fairweather 1989). The problem arises when we can measure 'recruitment' but know nothing about larval supply. If we are interested in the relationships between available larvae, settlement and eventual recruitment, it is important to measure each event as it happens, before patterns are obscured by early post-settlement mortality (Gaines and Bertness 1993, Hunt and Scheibling 1997). This especially applies to fields such as fishery management and restoration, where managers need to make decisions potentially affecting valuable human resources at the population level. Management of fishery species typically has been guided by models where mortality is often assumed to be constant through the adult phase based on measurements from large adults. The larval phase is generally treated as unimportant in modifying recruitment because it is assumed to occur before compensatory mortality ensues (Hunt and Scheibling 1997). Management plans have not been effective in preserving a fishery for long-lived, slow growing species like

abalone. Although some fisheries, including that for the Maine lobster, are still viable after over a century, size of individuals at harvest are substantially smaller and there are fears of eventual collapse (Steneck 1995). In response to the growing awareness of this widespread problem, Congress amended the Magnuson-Stevens Fishery Conservation and Management Act in 1996 to require assessment of the health of various stocks, and develop and implement rebuilding plans for those stocks whose biomass has declined to a level where they are considered "overfished." The failure of many regulated fisheries world-wide suggests that both the larval and juvenile stages may be important in determining the future population, and that early juvenile mortality is more important than previously believed (Caley *et al*. 1996).

The California red abalone (*Haliotis rufescens*) fishery was closed in 1997 by California State Senate Bill 463, after precipitous declines in stocks. The majority of the recent fishing effort had been based within the Santa Barbara Channel. Egg-per-recruit models suggested that commercial take, based on a minimum size limit, was still within sustainable limits (Tegner *et al*. 1989), but populations continued to decline. Although red abalone are still common in northern California (K. Karpov, pers. comm.) there is little chance of such areas effectively reseeding depauperate fishing grounds more than 500 miles to the south, when the predominant near-shore current direction is south to north.

In the chapters that follow, I describe my efforts on a red abalone fishery enhancement project. To test the feasibility of enhancing wild abalone populations by releasing farm-raised competent larvae into the field, I examined factors and

mechanisms that could affect settlement, survivorship, and growth. Initial work is detailed in Chapter Two, where I investigate larval behavior and settlement under a variety of laboratory conditions. I test larval choice of settlement on natural versus a variety of artificial substrates, and also examine settlement under different flow regimes. In Chapter Three, I outline the extensive field work that followed to assess natural post-settlement densities, the effects of water motion on larval supply, post-larvae survivorship up to three weeks after settlement, and the role of seasonality in survivorship and growth. In Chapter Four, I examine the role other species may play in abalone settlement and recruitment. I describe an experiment that examines the role urchins may play both directly and indirectly in abalone settlement success, then describe the results of field surveys for new abalone recruits at San Miguel Island and finally explore the possibility of using other newly-settled invertebrates as a proxy for good abalone habitat.

Historical Background

Abalone (Genus *Haliotis)* are dieceous broadcast spawners, releasing up to one million eggs at a time (Cox 1962). What triggers spawning remains unclear; researchers have implicated typhoons (Sasaki and Shepherd 1995), unusually calm conditions (Breen and Adkins 1980), or cessation of normal current regimes. Harvesting by commercial divers often induces release of gametes, both under water and within the live wells ship-board (pers.obs.). After fertilization, larvae continue to develop as free-swimming plankton for about a week, when they become

developmentally ready for settlement and metamorphosis. After release, abalone eggs are negatively buoyant and sink to the bottom. Upon hatching, larvae become weakly positively phototactic, and may begin limited 'non-directional' swimming. Although the larval stage lasts for at least a week, with the potential of dispersal on the order of tens to hundreds of kilometers, laboratory behavior suggests dispersal distances may be much more limited. Larvae may swim upwards for a few centimeters, and then slowly sink to the bottom (pers. obs.). Although this process has been extensively documented for many species of abalone in the laboratory (Morse *et al*. 1979, Ebert and Houk 1984), it becomes much more difficult to observe in the field. Such behavior may be a means for briefly getting the larvae back up into the water column, to move on a small scale from areas of poor habitat, and not a mechanism for long-term dispersal. It has been shown that chemotaxis may provide the cues needed for settlement (Morse *et al*. 1979) but these surface cues are only detectable by crawling, not swimming, larvae. If a larva, in contact with the bottom, does not receive the right cue, then it may employ a few seconds or minutes of swimming to lift off from the bottom and be carried by currents to a new area. On the scale of a larva, this distance need not be very far. However, evidence exists for *H. discus* that the intensity of the storm events that induce spawning in this species may also determine the scale of larval dispersal (Sasaki and Shepherd 1995). Additionally, Shepherd *et al.* (1992) showed that, for *H. laevigata,* recruitment varied spatially according to local coastal topography. More compelling, though, is the hypothesis that the apparent absence of a strong dispersal mechanism indicates that

settlement may be most prevalent, and successful, near the natal reef and conspecifics. This idea is also supported experimentally by Prince *et al*. (1988), McShane *et al*. (1988) and McShane and Smith (1991). So, given this sort of behavior and potential larval 'choice', what makes settlement successful? We may break this down into 3 components; larval *supply,* settlement *substrate,* and degree of *mortality* of post-larvae.

The initial *supply* of competent larvae depends in large part on the density of spawning adults. For a successful spawn to occur, adults must be densely aggregated, allowing high sperm densities and resulting fertilization (Clavier 1990). Babcock and Keesing (pers. comm.) have found that for *H. laevigata*, fertilization drops to 50% at a distance of 2 meters from the spawners, regardless of current conditions. Prince *et al*. (1987) demonstrated for *H. rubra* a strong link between density of recruitment and immediate density of spawning adults. Unfortunately for red abalone, at least in southern California, decades of sport and commercial fishing have reduced adult densities to less than 1 per hectare at Santa Rosa Island (K. Karpov, pers. comm.) and recruitment seems to be poor at best (Tegner *et al*. 1989, pers. obs.).

Primary settlement *substrate*, as demonstrated in the laboratory (Morse *et al*. 1979) and from the presence of newly-settled individuals in the field (Shepherd and Turner 1985), appears to be the crustose coralline species *Lithophyllum* spp. and *Lithothamnion* spp. Other benthic invertebrates also preferentially settle upon crustose corallines, such as chitons (Barnes and Gonor 1973) and the seastar *Acanthaster* (Keesing and Halford 1992). Shepherd and Turner (1985) examined

experimental boulders to verify observations of substrate preference by *H. laevigata*. They found that post-larvae up to two weeks old were largely restricted to crustose corallines. Moss and Tong (1992) found that under laboratory conditions, *H. iris* larvae almost exclusively settled on crustose coralline algae rather than on bare rock or diatom-filmed acrylic plates. Other studies have reported some laboratory settlement on bacterial and diatom films (Kaspar and Mountfort 1995, Tong and Moss 1992) and conspecifics' mucus trails (Slattery 1992). Although some invertebrate larvae are sensitive to water-borne chemical cues (Zimmer-Faust and Tamburri 1994), abalone larvae are reported to respond only at the source of an inducer (Morse and Morse 1984, Barlow 1990). Although much is known about the chemical processes of larval abalone settlement under laboratory conditions, extending the implications of these observations to the field is difficult. Morse (pers. comm.) points out that results showing larvae to be receptive to specific chemical cues found only in crustose algae were from 'unnaturally' ultra clean bacteria-free conditions, and may not reflect settlement in nature.

Mortality may be the hardest aspect of settlement success to measure, but it is one of the most important (Gosselin and Qian 1997). The processes determining settlement success act on very small spatial and temporal scales, often unmeasurable in the field. Mortality may be due to unfavorable physical conditions, such as sediment burial or abrasion (Schiel 1993), or from lack of suitable substrate, thus leading to eventual starvation or predation. Work on larval bryozoans (Hurlbut 1991), urchins (Rowley 1989) and cnidarians (Keen 1987) has shown the importance

of substrate selection on post-settlement mortality. Most predation work has focused on juvenile abalone greater than 3 cm (1 year old), as they are easier to find in the field, and are available from hatcheries. However, estimating mortality/survivorship rates on the sole basis of counting older, larger juveniles, is misleading, as there is enormous potential for mortality to occur within the first few days or weeks after settlement (Gosselin and Qian 1997). Additionally, abalone may move to cryptic or crevice habitat for predator avoidance, where they become difficult to track (Shepherd 1986). Schiel (1993) concludes that initial mortality rates are quite high for juveniles of all species, and that mortality decreases with body size.

Other Enhancement Work

The question of whether to enhance wild abalone stock is a pressing one. Abalones have been in decline worldwide, and demand an ever increasing market price. Although California mariculture facilities produce an estimated 242,000 pounds valued at $3.1 million annually (UC Sea Grant, 1994 figures), they are no substitute for the loss of a lucrative fishery once valued at $15 million. Additionally, intensive farming of abalone to a marketable size is expensive, laborious and time consuming. Understandably, there has been considerable interest in augmenting wild abalone populations. These efforts fall into two categories: translocating or transplanting wild stocks from other areas (concentrating existing stocks), and outplanting cultured abalone into the wild (enhancing stocks). Both methods hope to accomplish the same end result- more abalone- but are supported biologically and

economically for different reasons.

1. Translocation/Transplanting of Wild Abalone

The California Department of Fish and Game (CDFG), the National Park Service, and various other agencies have undertaken numerous abalone enhancement experiments. Although some work occurred at San Miguel Island with red abalone, most work was done within southern California, where most of the commercial fishing effort and other human impacts have occurred, and concentrated on green (*H. fulgens*) and pink (*H. corrugata*) abalone. Typically, fishery-legal adults were collected, either by agency biologists, or purchased from commercial fishermen, and were concentrated at a new location. Areas were chosen either as "good abalone habitat", or because they were within designated fishery-closure zones, such as within Marine Refuges at Santa Catalina and Anacapa Islands. Animals were tagged and measured, and their growth and survivorship monitored over time. However, for the majority of such experiments, survivorship was poor. Failure was attributed to emigration, starvation, predation, or poaching. In 1956, CDFG biologists transplanted roughly 800 red abalone from San Miguel Island to Santa Catalina Island. Soon after, however, the red abalone's primary food source *Macrocystis* began to die off from unusually warm water. After a year, only a few surviving abalone were found. It is believed that they had survived only by feeding upon deep water *Pelagophycus* (Cox 1962). In 1983, CDFG transplanted large *H. corrugata* from San Clemente Island to Santa Catalina Island. In less than a year, only 22% of

the abalone could be accounted for, and losses were attributed to illegal take (Henderson *et al*. 1988).

Transplanting, although mostly unsuccessful, was undertaken repeatedly for a variety of reasons. Transplanting adults attempts to correct a direct human impact. Fishing pressure is greatest on aggregations of harvestable individuals as searching for more widely scattered abalone is not as cost-effective: most fishing effort is therefore placed in densely populated areas (McShane and Smith 1988, Prince *et al*. 1988). This leads to a landscape of smaller, less fecund individuals rather than groups of varied sizes. In this manner, harvesting pressure lowers localized stocks and may contribute to unsuccessful spawning, leading to eventual recruitment failure. If adults are present, but widely scattered, it makes biological sense to concentrate them, and by doing so allow spawning and recruitment to occur naturally again. Unfortunately, such aggregations of large abalone often prove irresistible to poachers. Transplanting wild adults eliminates the need to rely on the aquaculture industry to produce suitable numbers of healthy farm-raised abalone for outplanting at a low cost. Additionally, transplanting avoids the controversial issue of introducing 'farm' genomes (Gaffney *et al*.1996), even though genetic markers might make proof of successful enhancement that much easier.

2. Outplanting Farm-Raised Abalone

Another way to enhance wild stocks of abalone is to release farm raised individuals. Outplanting farm-raised juveniles has been popular worldwide, although

there is little quantitative evidence for its success. Mass-produced "seed" (small juveniles) has been routinely outplanted along the coast of Japan since the late 1970's. Reported estimated recapture rates of planted 8-45mm *H. discus* range between 12-51% (Kojima 1995). Releases of hatchery-raised *H. iris* in New Zealand have experienced survival after three years as high as 24% (Schiel 1992). Small *H. rufescens* (41mm average) released into artificial habitats at Santa Cruz and Santa Rosa Islands, California had survival rates of 32% by one year and 24% by two years (Davis 1995). Since most enhancement projects are designed to increase the number of harvestable adults, releasing larger individuals would mean less growing time in the field to reach minimum harvest size. However, these larger abalone are more expensive to purchase as seed than smaller ones. Additionally, they might be vulnerable to predation by sea otters and octopi even at this larger size (Hines and Pearse 1982). Smaller abalone are quicker and cheaper to produce, and so may be outplanted at higher stocking densities. These smaller sizes may find refugia faster and thus survive the acclimation period better, but whether or not this offsets the risk of mortality during growth to harvestable size is unclear. Kojima (1995) could not account for the fate of juveniles outplanted at less than 13mm. Schmitt and Connell (1982) showed that short-term survivorship was inversely related to size at outplanting. Saito, as mentioned in McCormick *et al*. (1994), found that larger juveniles survived the winter best, and so choice of size at outplant depended on the season. Additionally, non-cryptic behavior of hatchery juveniles puts them at a greater risk of predation than same size "streetwise" wild animals (Schiel and Welden

1987).

In the 1980's there were several large-scale outplants of farm-raised red abalone. Schmitt and Connell (1982) released 9,000 10-45mm hatchery juveniles onto Naples Reef, near Santa Barbara in 1980. Monitoring for 17 weeks after outplanting revealed approximately 1% mortality per week. After two years, however, there was no discernible enhancement of abalone in the experimental site. Mortality was attributed to environmental stress and subsequent starvation associated with strong storms that removed macroalgae from the reef. At the same time, researchers released 40,000 juvenile red abalone onto reefs at Tyler Bight, San Miguel Island. Although subsequent follow-up surveys were incomplete, recent genetic work on abalone harvested from this area during the 1990's suggests that much of the existing broodstock was very similar genetically, suggesting farm-raised seed (Gaffney *et al*. 1996). This discovery, combined with observations from commercial abalone fishermen, implicates the 1980 outplants or their progeny as being harvested abalone. Although useful as a marker, the genetic homogeneity of farm abalone raises the issue of whether it is a good idea to enhance wild stocks with virtually identical genomes. Some farms are aware of this problem, and always use broodstock from different origins when spawning (as in Hawn 1989). Another problem is that outplanting may alter natural patterns of genetic structure. Introduced, poorly-adapted genotypes could swamp adaptations to local conditions that have evolved over long time periods. Unfortunately, recent fertility problems among wild animals from southern California and a general lack of males state-wide

has put increasing pressure on using farm stock for spawning (Shane Anderson, pers. com).

3. Larval Seeding

Limited information exists on larval outplanting experiments. Although the concept of augmenting natural abalone populations with larvae has been discussed for decades, the inherent difficulty in finding and tracking larvae of any species in the field has discouraged extensive research. When compared to the costs of juvenile rearing, production to larval stages sounds very attractive. Researchers in New Zealand have shown that it is possible to find settlement within a week after release of competent *H. iris* larvae into isolated surge channels. The settlers were found in greatest numbers closest to the release site (Tong *et al*. 1987). Preliminary data from Schiel (1992) suggest that larval releases are not cost-effective when compared to survival of larger juveniles, and anecdotal information of work done in Baja California suggests limited discernible settlement and survivorship.

In 1991, the State of California together with the California Abalone Association (CAA) imposed a tax on abalone landings. This tax created a fund for research on fishery enhancement, and made the current research possible. I attempted to answer the question 'does larval outplanting work' by first exploring larval behavior and then performing a series of experiments designed to test various parts of the problem.

Field Site Selection

All field experiments took place on Isla Vista Reef (34° 24'150" N, 119° 51'620" W), a shallow (10-12m) area just offshore of Isla Vista and the University of California, Santa Barbara (UCSB) (Figure 1). The bottom is characterized by low-lying shale reef running parallel to shore, interspersed with sand and cobble patches. The reef remains fairly shallow up to 800 meters offshore, and thus inner areas often are affected by sand movement and particulate accumulation. *Macrocystis pyrifera* is locally abundant along rocky ridges, and forms a dense canopy excluding most bottom algae during the summer months. Seasonal algae, such as *Desmarestia lingulata*, can be very abundant over large areas. Much of the rock surface is covered with crustose coralline algae, primarily of the genus *Lithothamnion* spp. Common benthic macro-invertebrates include seastars *Pisaster gigantea* and *P. brevispinus*, the urchins *Strongylocentrotus purpuratus* and *S. franciscanus,* the sea cucumber *Parastichopus parvimensis,* the whelk *Kelletia kelletii,* the top snail *Lithopoma undosa,* and various boring bivalves such as *Pholus* sp. Occasional species include *Octopus* spp., the giant keyhole limpet *Megathura crenulata,* the lobster *Panulirus interruptus,* and crabs *Cancer* spp. and *Loxyrhynchos grandis.* Infrequent and rare taxa include both red abalone *Haliotis rufescens* and *H. corrugata,* the pink abalone. Common fish taxa are sheepshead *Pimelometopon pulchrum*, kelp bass *Paralabrax clathratus*, sand bass *P. nebulifer*, blackeye goby *Coryphopterus nicholsii*, and various surfperches. This reef was chosen in part due to its proximity to UCSB and because red abalone were historically common in this area (commercial fishermen,

pers. comm.) but now are at densities of less than 1 per 10,000 m^2 (Altstatt, unpub. data). The current scarcity of abalone likely reflects the heavy fishing pressure that reduced abalone populations throughout southern California, and not some unfavorable site characteristic. Therefore, since Isla Vista Reef was once good abalone habitat, success of settlement from released larvae should not be limited by habitat constraints. Additionally, low wild stocks ensure little, if any, natural recruitment, thereby allowing for proof of concept tests (i.e., any larvae recovered during the experiments are probably outplanted and not from wild spawns). After surveying most of the reef, a mostly flat rocky area, 12 meter depth, was selected. No abalones of any species were found within 400 meters of the site.

Chapter Two. The Importance of Biotic and Abiotic Factors on Larval Behavior: Substrate Condition and Flow Regime

Introduction

Settlement choice and the importance of substrate have been demonstrated for a variety of marine invertebrate larvae (Carlton and Sammarco 1987, Raimondi and Keough 1990, Walters 1992). In addition to the presence of attractive chemical signals (Morse *et al*. 1979), other cues influencing settlement may include the presence of already settled larvae and small-scale abiotic factors like surface rugosity (Maldanado and Uriz 1998). Abalone larvae, unlike barnacles, are not gregarious settlers. However, they often appear to settle preferentially in certain areas of coralline crust, often around small bumps (pers. obs.). This could be an artifact of flow conditions (they get washed off elsewhere) or the result of chemical cues or other physical features. This idea can be tested by offering larvae different substrates under varying flow regimes.

Water motion and flow are universal factors encountered by marine species, although their influence may vary with life-history stage (Roughgarden *et al*. 1988). Flow conducts chemical signals, mixes eggs and sperm, disperses larvae, and transports food. Depending on the species and breeding mechanism (broadcast spawn, brooder, crawl-away larvae), flow conditions could be crucial to successful settlement and recruitment (Abelson 1997, Eckman *et al.* 1989). For many years it was thought that since abalone are broadcast spawners, their propagules must be

carried by currents far away from the parent stock (Tegner and Butler 1985). This is certainly the case for many fishes and invertebrates such as lobsters, whose larvae are in the plankton for up to a year (Menzies and Kerrigan 1980). But once the larvae are ready to settle onto the substrate, flow could become a critical factor in determining whether larvae reach the bottom and search for settlement substrate (Eckman 1990). Abalone larvae are not strong swimmers, and it is possible that flow conditions could exist that prevent the larvae from settling, even if they recognize the chemical cues as they are carried past a suitable substrate (Abelson 1997). The ability to choose settlement substrate, or distinguish between live crusts and nearby dead crusts may change under differing flow environments (Pineda and Caswell 1997). However, crawling larvae may be able to effectively explore the substrate for cues under flow speeds greater than their swimming speeds (Abelson 1997, Walters 1992).

In the following sections, I describe a series of experiments to determine if (1) larvae choose between settlement surfaces in still water, (2) larvae can distinguish between live crusts and nearby dead crusts under different flow regimes, and (3) once crawling on the bottom, if larvae can effectively explore substrate for cues under flow speeds greater than their swimming speeds.

Methods

2.1 Settlement Behavior in Still Water

2.1.1 Larval Settlement Behavior- Substrate Choice

This experiment explored the hypothesis that dead coralline crusts were as

inductive as fresh crusts, and that competent larvae would not settle preferentially between them. Additionally, the ability of bleached crusts to induce settlement was tested as well. Three types of settlement surfaces were prepared: live, dead and bleached. Flakes of living coralline algae were scraped from a flowing seawater tank and then affixed (using superglue) to glass slides. Dead crusts were prepared in the same manner, but using dried coralline algae scraped from a seawater tank that had been empty for several months. These slides were placed in seawater as soon as the glue had set, and soaked for at least an hour. Bleached crusts were prepared by drying living crust-covered glass slides in a drying oven for two days, and then in direct sunlight for one day. To test larval settlement on each surface, one live and one dead slide was placed in each of two glass finger bowls. A third bowl contained only a live slide, a fourth only a dead slide. A fifth and sixth bowl each contained one 'bleached' slide. All bowls were filled with filtered seawater. Approximately 50-100 competent larvae were added to each bowl, which was then placed in a cooler (16° C) for twenty-four hours. I assessed settlement by examining each bowl with a dissecting microscope and counting larvae and noting condition.

2.1.2 Settlement as a Function of Exposure Time

I designed this experiment to test the hypothesis that larvae are more likely to settle immediately upon introduction to a surface, and that larvae are no more likely to settle more quickly on live crusts than on dead crusts. All trials took place in plastic culture trays, each with six shallow wells. Each well contained a single piece

of coralline algal crust, either 'live' or 'dead'. Live crusts were chipped off the side of a flowing seawater tank. Dead crusts were removed from a tank that had stood empty for an undetermined amount of time, but long enough for the coralline algae to become bleached in color. Dead crusts had all of the same characteristics as live crusts except for living tissue. Chips were approximately 1-1.5 cm^2 in area. Wells were filled with filtered seawater. Approximately 20 competent larvae were dropped from a pipette into each well. Trays were left partially covered on a lab counter during the experiment. Three trays (9 wells of each crust) were sampled at each of five time intervals; 2.5, 6.5, 25.5, 53.75 and 73 hours after introduction of larvae. Sets of trays were used for discrete time intervals and were not re-sampled. The fate of each larva was scored by examination under a dissecting microscope. Three categories were used to describe larval condition; settled, swimming, or dead. Larvae were scored as settled if they were upright and actively moving about, and the velum was missing. If the velum was obviously still attached, the larva was scored as swimming. The data for this experiment were analyzed using a single factor ANOVA for each time period with crust type as the factor.

2.1.3 Coralline Crust Substrate: Role of Algal Films

This experiment was designed to test if diatom films (inductive for some species) masked the desirability of coralline crusts. Settlement trials took place in flowing seawater tanks at the UCSB Marine Laboratory. The experimental substrate was sanded Plexiglass plates (10cm by 10cm) that had been cultured in a shaded

flowing seawater tank for one year, during which time they had become covered with the encrusting coralline algae *Lithophillum*. To test larval settlement success as a function of substrate condition, I prepared two conditions ('algal film', 'no algal film') in the following manner. I placed encrusted plates into separate Tupperware containers. 'Unfilmed' plates (n = 6) were incubated in a shaded flowing seawater tank. Shaded conditions prevent overgrowth of diatom films on these plates. Additional plates were placed into an uncovered tank. The higher light level in this tank allowed for diatom growth, and these plates are referred to as 'filmed' (n = 4). All containers and plates were left for a week. All containers were then placed on a table and flushed with fresh seawater. Flow was stopped so that the water level in each container was just below the top. Abalone larvae were obtained from a commercial mariculture facility (The Cultured Abalone, Ellwood, CA) and were a week past the onset of competency. Approximately twenty larvae were pipetted into each container. After two days, each plate was carefully removed and examined under a dissecting microscope, and all abalone were counted. The data for this experiment were analyzed using a single factor ANOVA.

2.2 Settlement Behavior in Flowing Water

To examine the ability of abalone larvae to settle onto substrate under a variety of flow regimes, the following flow experiments were run in a recirculating 38 L Plexiglass flow tank. A baffle was situated at the head of the tank to initiate laminar flow. The tank was fitted with a false bottom into which slide-shaped

depressions were milled. Slides placed into these slots sat flush with the bottom of the tank. Slides were arranged into two rows of three across, with treatments staggered. The tank was flushed with fresh seawater between each trial. The tank was fitted with an electric variable-speed submersible pump, controlled by a finger knob. The knob was labeled from 0 to 10. To calibrate the pump, flow speeds were calculated by the distance traveled by a neutrally-buoyant object over a marked distance, for each level on the control knob.

2.2.1 Crusts versus Bare Glass Substrate

This experiment tested larval choice of settlement substrate under four flow speeds (0, 3, 7 and 13 cm/sec). These speeds were chosen as representative of a range of natural conditions. Four consecutive 24 hour trials were run. The order that the trials were run is as follows: Day 1 speed = 3 cm/sec, Day 2 = 13 cm/sec, Day 5 = 7 cm/sec and Day 6 = 0 cm/sec. The two treatments (frosted glass slides n = 3, slides covered with crustose coralline algae n = 3) were staggered on the bottom. Larvae were obtained from Daniel Morse's laboratory, and were 1 day past competency at Day 1. For each time trial, approximately 100,000 larvae were placed in the tank and allowed to circulate for 24 hours. After 24 hours, flow was stopped and the slides were carefully removed and examined under a microscope. Larvae were scored as settled if they were attached, upright or crawling, or "stuck" if they were clumped together, upside down, or not obviously settled. The data were analyzed using a one-way ANOVA with flow as the factor.

2.2.2 Settlement Choice Under Different Flow Speeds

This experiment was designed to examine larval choice between live crusts and nearby 'dead' crusts under a variety of flow conditions, and to test the hypothesis that settlement success changes with age after competence. Larvae were obtained from the UCSB Morse hatchery facility. The larvae became competent two days before the experiments began, and were kept under normal culture conditions until needed. 100,000 larvae were used for each trial. Two treatments of settlement substrate were used for each trial; 'live' coralline algae slides (n = 3) and 'dead' bleached coralline algae slides (n = 3). Crusts were super-glued to glass slides as before. Consecutive 24 hour trials were run with speeds ranging from 0 to 25 cm/sec (0, 5, 10, 15, 20 and 25 cm/sec). Speeds were assigned to each trial randomly. For 0 cm/sec, I briefly turned the pump on while adding the larvae to ensure thorough distribution throughout the tank. Trials were referred to by the age of the larvae on that particular day. Days 6 and 8 were discarded due to equipment problems. On Days 11 and 12, I repeated speeds of 0 and 15 cm/sec to control for age effects. At the end of each trial, slides were carefully removed from the bottom one at a time, placed into a petri dish and scored for larval condition using a dissecting microscope. To control for potential day effects, I standardized the data by combining settlement estimates for live and dead crusts into one proportional variable, the number of larvae settled on living crusts divided by the number of larvae settled on live crusts plus the number of larvae settled on dead crusts. I used this proportion as an index of larval

condition since previous trials showed that 95% of larvae settle on living coralline crusts rather than dead if given a choice.

To determine if there was an effect of larval age (day effect), I ran an ANCOVA with speed and day as factors (day was the covariate). This analysis was run on the arcsine transformed proportion of larvae that settled on live crusts.

Results

2.1. Settlement Behavior in Still Water

2.1.1. Larval Settlement Behavior- Substrate Choice

When larvae were given a choice of settlement surfaces, 95% settled on live coralline crust, and the remainder on dead crusts (Fig. 2.1.1). All larvae settled in the 'live crust only' treatment, whereas only 9% of larvae settled in the 'dead crust only' bowl and swimming larvae were still observed. No larvae were observed to be still swimming in any of the other bowls. All of the larvae in the 'bleached crust only' bowls settled.

2.1.2. Settlement as a Function of Exposure Time

Larval behavior varied both over time and with substrate surface. Significantly more larvae settled when exposed to live crusts than dead crusts over all time periods up until 73 hours (Fig. 2.1.2, Table 2.1.2).

There were significantly more larvae swimming in the wells with dead crusts than those with live crusts for all time periods. On average, 66% of larvae were still swimming after 2.5 hours in the dead crust treatment, compared to 27% in the live

crust treatment. In both treatments, the number of swimming larvae declined with time.

Larvae began to die by 25.5 hours, but there was no significant difference in the number of larvae dying when exposed to either crust type over all time periods ($P = 0.649$, $F_{1, 88} = 0.207$).

At 2.5 hours, most of the larvae in both treatments were either swimming or settled (Fig. 2.1.2). From 6.5 hours on, almost 90% of larvae in the live crust treatment were crawling, compared to less than 50% of larvae in the dead crust treatment. At 73 hours, however, the number of crawling larvae in the dead crust treatment increased to 66%. Significantly more larvae were crawling in the live crust treatments than in the dead crust treatments at any given time ($P < 0.0001$, $F_{1, 88} = 107.468$).

2.1.3. Coralline Crust Substrate: Effect of Algal Films

The presence of a thick diatom film which covered the coralline crusts appeared to interfere with settlement (Figure 2.1.3). Settlement was low on filmed (2.5%), and high on unfilmed plates (48%). The difference in settlement between these two treatments was highly significant ($P = 0.001$, $F_{1, 8} = 22.704$).

2. Settlement Behavior in Flowing Water

2.2.1. Crust versus Bare Glass Substrate

Larvae were scored as settled if they were attached, upright or crawling, or 'stuck' if they were clumped together, upside down, or not obviously settled.

Settlement on crusts was not affected by flow speed (Table 2.2.1, Figure 2.2.1). A mean of 12 larvae settled on crusts at all flow speeds. The fraction of individuals with shells edges stuck to crusts was greater at higher flow speeds (ANOVA, $P = 0.031$, $F_{3, 8} = 4.982$), with more than twice the number of larvae apparently stuck. No larvae were found settled on the frosted glass slides, and those 'stuck' to glass did not change significantly with flow.

2.2.2. Settlement Choice under Different Flow Speeds

Significantly more larvae settled on live crusts than on dead crusts for all trials ($P < 0.001$, $F_{1, 46} = 46.026$; Fig. 2.2.2). Unlike the previous flowtank experiment, all larvae that were on the substrate appeared to be settled and not 'stuck'.

There was no significant effect of larval age or flow speed on the proportion of larvae settled on live crusts (Table 2.2.2). The most striking observation was an increase in settlement on Days 11 and 12. Settlement on both of these days was higher by an order of magnitude than any of the preceding days; however, as there was also higher settlement on dead crusts, there was no significant change in the proportion of larvae that settled on live versus dead crusts.

Discussion

By the time that abalone larvae reach competency in nature, currents may have carried them from the natal reef and away from conspecifics. The ability to quickly recognize good settlement substrate that contains the chemical cues needed

for metamorphosis is critical for survival, and an individual larva might not get more than one chance to settle. Conversely, larvae may experience repeated exposure to settlement surfaces throughout their competency period (Pineda and Caswell 1997). The above experiments explored changes in settlement patterns over time when larvae are exposed to different surfaces, and under various flow regimes.

Results of my settlement experiments were consistent with previous findings that abalone larvae use chemical cues in coralline algae for settlement (Morse and Morse 1984). The different surfaces provided measurably different settlement responses over a short period of time. Larvae settled preferentially on live coralline algal crusts. The dead crusts used in the finger bowls were not inductive, even when they were the only available substrate. Settlement on dead crusts did not increase in the 'choice' trial, and after 24 hours, 95% of larvae remained upon the live crusts. Since after exposure to both surfaces for 24 hours, most larvae ended up on the live crusts, these results support Morse *et al*. (1979) who showed that the chemical cue was only available to crawling larvae, in direct contact with the surface substrate.

Surprisingly, settlement on bleached crust was just as good as live crust. Even after three days of drying, the bleached coralline crusts were still highly inductive. Although the bleached coralline algae were not observed to recover after the experiments, it is unlikely, but possible that the alga was actually still alive. Under natural conditions, it is possible that larvae could encounter bleached crusts after a very low spring tide, or during active overgrazing by urchins. Bleaching can also occur following sand burial, which is a common occurrence along the edges of low-

lying reefs. Accumulations of drift algae along such reef edges make these areas attractive to abalone. Larval recruitment often occurs near aggregations of adults so it is possible that larvae encounter bleached crustose algae in the natural environment.

In contrast with initial trials, dead crusts became more inductive over time. It is possible that the tank they were scraped from had not been empty long, and that re-exposure to seawater leached out chemical cues. More likely, however, is that bacterial growth was high enough by 73 hours to induce settlement. Morse and Morse (1984) have shown that exposure to certain chemicals while the larvae are still swimming in culture can augment settlement once an inducer is encountered. I had expected settlement on dead or bleached crusts to be minimal. However, almost 70% of larvae in the dead treatment were actively crawling after 73 hours. Swimming larvae often cease moving and fall to the bottom, where after a period of testing, they either may resume swimming in the absence of an inducer, or proceed to shed the velum and begin metamorphosis. Once settled, larvae continue to explore the surface and aggregate around certain small-scale features that might increase chances of survival (pers. obs.). Larvae were not re-examined to check for actual completion of metamorphosis, and so these data can be only used to elucidate pre-settlement behavior.

Larvae settled preferentially on coralline crusts under all flow speeds, and no larvae were found settled on the bare glass slides. In the first flowtank experiment, I discovered a tendency for larvae to be clumped or stuck together. At lower flow, this number was not different from the larvae that appeared to have settled, but at higher

speeds the number of stuck larvae increased significantly. As the larvae tested at speeds of 7 cm/sec were twice as old as those tested at 3 cm/sec, it is possible that susceptibility to sticking and clumping is an effect of larval age, but this trend was not observed in settlement or stickiness on glass substrates. Many larvae were observed to be stuck or lodged in the crevices around and behind the recessed slides, most likely as a result of small-scale hydrodynamic forces. However, this stickiness did not appear to increase settlement. It is possible that the surface topography of coralline crusts created better conditions for larvae to settle on, or perhaps the glass, although frosted, was too slick. If larvae stuck to the substrate merely due to aging or current speed, they should have stuck equally to crusts and glass. However, larvae stuck to crusts seven to ten times more often than glass. Either the rugosity of the coralline surface trapped moribund larvae, or else they initially preferentially settled on the crusts and then became stuck. It is also possible that the entire batch of larvae were physically stressed, as none of the larvae in the next flow tank experiment showed any signs of being 'stuck'.

If larval delivery affects settlement, and thus at higher speeds the larvae would have many more chances to pass over the substrate, settlement in flowtanks should increase with speed. However, settlement was similar throughout the range of speeds tested. A possible explanation for this pattern could be that at faster flow speeds, although there is more exposure to settlement surfaces, larvae are unable to physically settle any faster than at lower speeds. The mid-water current may be too fast to allow larvae to sink to the bottom as they pass over the settlement surfaces. In the flowtank,

even though the delivery rate increased, an increase in small-scale turbulence flow at higher speeds might have prevented an increase in physical attachment. However, as shown by Walters (1992) both barnacle (*Balanus)* and hydroid (*Bugula*) larvae settle preferentially at the bases of small bumps, where flow is the greatest.

In the second flowtank experiment, settlement was an order of magnitude higher when I repeated trials of 0 and 15 cm/sec. (Figure 2.2.2). The larvae were eleven and twelve days past the onset of competence at this point, which appears to have caused a major increase in settlement. However, the proportion of settlers on live to dead crusts did not change significantly over the course of the experiment, and the statistical analysis did not reveal any speed or age effects. At this age most larvae, although active, are no longer swimming (personal observation). Some researchers suggest that older larvae go through metamorphosis more quickly than do younger larvae (Moss and Tong 1992). As larval yolk supplies run low, the weakening larvae may become less 'picky', or perhaps less of a cue is required to induce settlement. However, while initial settlement of older larvae may be greater, anecdotal observations suggest that survivorship through metamorphosis may not (Pechenik 1990).

Finally, were the flow speeds in my experiments representative of natural conditions? When I ran the experiments, it was difficult to control flow speeds on a fine scale, especially when under 10 cm/sec. Eckman *et al*. (1989) reported mean current speeds 1.5 meters above a rocky reef ranging from 15-24 cm/sec, depending on density of understory kelps. Mid-water currents may often reach 30 cm/sec, but

larvae near the bottom are more likely to experience much lower speeds. Walters (1992) found that stresses on *Bugula* larvae at flow speeds of 15 cm/sec were an order of magnitude lower than those determined by Eckman (1990) as critical levels needed to dislodge larvae. It is possible that even at the fastest flow speeds, abalone larvae were still able to exert behavioral choice.

Chapter Three. Larval Settlement, Post-Settlement Survivorship and Recruitment of the Red Abalone *Haliotis rufescens* as a Function of Larval Supply: A Field Test

Introduction

Little information exists about natural settlement of abalone larvae. Extensive laboratory work suggests high variability in larval survival and settlement success (Hahn 1989), but whether these findings can be extrapolated to field conditions is unclear. Understanding the relationship between available settlers and a surviving cohort is important not only for answering questions on an ecological level (Minchinton and Scheibling 1991), but also for planning fishery or population enhancement schemes (Prince *et al.* 1988). A large female red abalone can release millions of eggs during a spawning event (Tutschulte and Connell 1981); given this high fecundity and apparently low juvenile and adult densities, failure of an individual larva to recruit seems highly probable. The relationship between high fecundity and low juvenile survivorship is well known in other species (e.g., rockfish) and may imply potential difficulties in releasing cultured abalone larvae as a means of augmenting wild populations. For abalone enhancement, a key issue to understand will be how the probability of a larva surviving past settlement varies (if at all) with the density of released larvae. Some additional factors affecting larval settlement and survival in the field may include delivery of larvae to the substrate, actual amount and

condition of the substrate itself, predator density, and condition of the physical environment (Gaines and Roughgarden 1985, Raimondi 1988).

I addressed these issues through a series of field experiments where I provided all of the competent larvae available to settle in a given area, thereby accounting for larval supply. Controlling initial larval densities allowed me to assess mortality or settlement success more confidently than if I did not know the original potential. This unique opportunity eliminates the uncertainty found in many supply-side ecology experiments (Gaines and Bertness 1993).

I designed the following field experiments to help explain some of the natural variability in settlement and subsequent survival. My experimental design allowed me to examine settlement repeatedly under similar conditions. Delivery of larvae to the substrate and actual amount and condition of the substrate was controlled in each experiment. When feasible, I also considered predator density and condition of the physical environment. I could not control for differences between batches of larvae obtained from the hatchery and small-scale environmental fluctuations. In the following sections I discuss first the results of surveys of natural recruitment at San Miguel Island, to assess natural conditions and provide comparisons for the results of my experiments. I then discuss a series of field experiments where I examined the density of released larvae required to detect settlement, the difference in settlement and recruitment success when larval supply was controlled, and variation in settlement and recruitment between seasons.

Methods

3.1. Survey of Natural Recruitment at San Miguel Island

I quantified natural densities of small post-settlers in the field to provide comparative data for my field experiments. After searching for appropriate sites along the mainland, I chose San Miguel Island as a standard for comparison as it is a highly productive and pristine environment. Most importantly, however, San Miguel Island was the only location in Southern California where recruitment of red abalone was still common after 1996. Surveys were done in November 1997, at Wykoff Ledge (on the south side of the island), in areas that appeared favorable for recruitment, such as rock faces and crevices adjacent to kelp beds supporting a healthy adult abalone population. Density of wild recruits and juveniles was estimated by searching 13 randomly placed 0.25 m^2 quadrats and counting all post-larvae and juvenile abalone. I was unable to adequately search cobble habitat or additional sites due to poor diving conditions.

Field Experiments

All larval release experiments took place on Isla Vista Reef (as described in Chapter 1). To provide replicate sampling areas, twenty plastic milk crates (30 cm x 40 cm x 30 cm, 0.11 m^2 in bottom area) were fixed to the rocky reef (Figure 3.0). Anchoring eyebolts were set in drilled holes with marine epoxy. Additionally, each crate was weighted with 9 to 12 kgs of lead in a sealed container, and was separated from neighboring crates by three to twenty meters.

Preliminary trials with a variety of settlement substrates indicated that using naturally-occurring cobble rock was the best option. Although Plexiglass plates ensured identical replicate surface areas, I found that the lack of micro-scale topography contributed to high losses of settlers. Settlers were more vulnerable to scouring, abrasion and predation on artificial plates (pers. obs.). Although natural rock potentially harbored terebellid polychaetes that prey on larvae (Morse *et al*. 1979), I decided to use this readily available, abundant substrate. Additionally, cobble has been shown to provide nursery habitat for wild juvenile red abalone up to a few centimeters in length (Morse *et al*. 1980, Ault and DeMartini 1987, pers. obs.) as well as refuge from predators (Schiel and Weldon 1987).

Crustose coralline algae-covered cobble rocks, ranging in size from 5 to 10 cm in length, were collected from a nearby area. I chose rock from a shallow (5m) section of reef, as Morse *et al.* (1979) found that terebellids were less common on shallow reef crests than in deeper offshore or inshore areas. Cobbles were chosen for similarity in general shape and size and abundance of crustose coralline algae. Cobbles were brushed lightly to remove any noticeable epibionts or sediment, and any cobble obviously harboring predators (e.g., terebellids) was discarded. A sub-sample of one or more collected rocks per crate was examined by microscope; no abalone recruits of any size were observed. For all experiments, each crate received twenty similar cobbles. Surface area of cobbles in each replicate crate varied but approximated 1500 cm^2; however, for the purposes of these experiments, area is given as bottom area, not total surface area.

3.2. The Effects of Supply Density on Larval Delivery and Settlement

To determine the density of larvae required to offset immediate losses from currents, and to understand my ability to detect settlement, I released larvae into experimental units in densities spanning three orders of magnitude. Crates (n = 5 for each treatment) containing natural cobble substrate were randomly chosen as replicates for each treatment: Low (two thousand larvae), Medium (twenty thousand larvae) or High (two hundred thousand larvae). Additionally, five crates were used as controls and did not receive any larvae. Larvae were obtained from Daniel Morse's hatchery at UCSB within a day after the onset of competency, and were transported to the site in 20 liter buckets. Shipboard, the larvae were split into replicates of the desired density. Larvae were released from sealed containers underwater directly over the baskets of cobble. At days one and nine after seeding, I carefully removed one cobble from each crate and immediately sealed it within a Ziploc baggie. At day twelve I repeated the sampling but with three cobbles per crate. At Day 33, I sampled all of the remaining rocks from the High density treatment; unfortunately I was unable to sample the remainder of the crates at this time.

For all sampling dates, after returning to the lab I carefully examined each cobble under a dissecting microscope, illuminated by a fiber-optic lamp, and counted the number of abalone settlers.

3.3. The Effects of Water Motion and Supply on Settlement Density

Due to the low numbers of settlers observed from the larval delivery experiment, it seemed possible that a high percentage of larvae was swept from the area by currents or water motion before they were able to settle. To address this, I devised an experiment where I released larvae either into crates as before, or into bagged (tented) crates which were sealed for 24 hours, to allow for maximum settlement. This way I could quantify initial losses of larvae, possibly resulting from water motion.

Larval Release Procedure

The experiment began on December 7, 1995. Larvae were obtained from Daniel Morse's hatchery at UCSB and The Cultured Abalone mariculture facility (Ellwood, CA) within a day after the onset of competency, and were transported to the site in 20 liter buckets. Shipboard, the buckets of larvae were split into 20 equal containers, with approximately 40,000 to 50,000 larvae in each. These were then filtered through a 250 micron screen to remove most of the water, and carefully poured into 60mm syringes. Each syringe was topped off with seawater, and the stopper inserted. I carefully bled each syringe before I took them underwater to remove any air pockets.

Half of the crates were randomly chosen to be tented. These crates were entirely enveloped within 'larval retainment' (Hefty) bags immediately prior to release of larvae. To release the larvae, I carried syringes underwater a few at a time.

I carefully approached each crate so as not to stir up the sediment or create eddies, positioned myself immediately over a crate, and then slowly pushed the plunger into the barrel. The concentrated larvae were easy to see as they spilled out onto the cobbles. While releasing the larvae I was always careful to move the syringe over the entire crate. I continued to dribble larvae out of the syringe until it was almost empty, whereupon I drew water back into the syringe to suspend any larvae that might have been crawling on the sides. Once I had rinsed out the syringe several times, I slowly backed away until I was confident that my motion was not disturbing the water around the crate.

To release larvae into a tented crate, I grasped the mouth of the bag and, sticking the syringe into the bag, held it tightly closed around my arm while I pushed in the plunger. After release, I slowly pulled my arm out and then quickly tied the mouth of the bag shut. I continued this procedure until tented (n = 9) and untented (n = 7) crates were seeded. Increasingly bad sea conditions prevented me from seeding all twenty crates as planned.

After 24 hours, I returned to the site to remove the tents and to sample for initial settlement. At each tented crate, I carefully cut the tent off at the base. When I removed the tents I did not observe any larvae in the water. Then, for all crates, I carefully removed all of the cobbles from one half of the crate (approximately 10) and immediately placed them into labeled Ziploc baggies. Cobbles were selected from one side of the crate only so as not to disturb the remainder. The bags of cobble were carefully brought back up to the boat and then immediately placed into buckets of

seawater. Any bags that were obviously leaking were double bagged. All samples were immediately brought back to the laboratory. On the second day after seeding, I returned to the site and repeated the collection procedure for the remaining 10 cobbles in each crate.

In the laboratory, the contents of each bag were poured into a tray of warm freshwater. Additional water was used to thoroughly flush out the inside of the bag. Enough water was in the tray so as to completely cover the cobbles. Cobbles were allowed to sit for approximately fifteen minutes while the freshwater acted as an anesthetic. Each cobble was then rinsed with a stream of water over a 300 micron sieve. Then, the water remaining in the tray was poured through the sieve and the tray was rinsed as well. All material in the sieve was then rinsed into a plastic vial and preserved with 70% ethanol. This procedure was repeated for each sample.

Under a dissecting microscope, all abalone from each sieved sample were removed with a glass pipette and placed into separate glass vials filled with 70% ethanol. Abalone were then counted, and a random subset was measured using a micrometer under either 12x or 25x power. Shells were measured across the longest side.

Settlement at Day 1 was assessed as the fraction of larvae found / larvae released. Survivorship at Day 2 was assessed as the fraction of Day 2 settlers / Day 1 settlers. The data were analyzed for each treatment with a one-way ANOVA with day as the factor.

3.4. Seasonal Differences in Settlement and Recruitment Success

To address the idea that seasonal differences in water temperature could affect settlement, I examined larval settlement success, survivorship and growth over the time span of a month, during the Spring (cold water) and the Fall (warm water) seasons.

I used the methodology outlined in the previous section, but all crates (n = 15) were tented for the first 24 hours. In both Spring and Fall, individual crates were sampled at one day, seven days and 28 days after release of larvae. Two Spring day one samples were damaged and therefore were not scored. After 28 days, all crates were left with half of the cobbles remaining. During the Spring experiment, all cobbles from four crates were removed for sampling at 15 weeks, and at 20 weeks, all cobbles remaining in each crate and the empty crates themselves were visually inspected *in situ* and any abalone observed were measured. For the Fall experiment, all crates were visually field surveyed only, at 10 weeks after release. Strong December storms prevented further sampling after this date.

After collection of cobbles, samples were processed as previously described. Additionally, when sorting the samples, I noted the proportion of empty shells to those containing live individuals. Shells containing tissue with black eye spots were considered to be alive when sampled. Empty shells were considered to be an indication of recent predation within that sample, and the proportion of empty shells found in each sample was used at the estimate of predation rate.

Settlement was calculated as the number settled as a percentage of the number

released. Survivorship was calculated as the number recovered after a certain time as a percentage of those settled after 24 hours. Growth of settled abalone was calculated by measuring all shells recovered from treatments on the longest axis.

Data (number of abalone recovered from each time period) were analyzed for each treatment with a one-way ANOVA with day as the factor. I chose a one-way ANOVA because I wanted to examine the differences between specific time intervals in each season.

Results

3.1. Natural Recruitment at San Miguel Island

All juvenile abalone observed at Wykoff were found beneath red urchins. Recruits fell into three distinct size classes (Figure 3.1), with 76% of recruits between 1-4 mm. Overall mean density of recruits was 4.77 per m^2. I found recruits in over half of the quadrats. Four of the quadrats were situated in a slightly deeper (calmer) area and contained no abalone.

3.2. The Effects of Larval Supply Density on Settlement

Although the greatest number of larvae were found after one day in the high density cobbles (Figure 3.2), there was no significant difference in number of larvae recovered per cobble between the high density and medium density treatments at any time ($P = 0.386$, $F_{1, 28} = 0.844$). No larvae were found in any of the low density rocks, with the exception of 2 individuals that were sampled on Day 12. One larva

was found on a control cobble at Day 12.

3.3. The Effects of Water Motion on Settlement and Survivorship

Tenting crates greatly enhanced initial settlement. There was significantly higher settlement ($P < 0.001$, $F_{1,14} = 17.739$) at 24 hours in tented crates (mean = 2,536, n = 9) than untented crates (mean = 71.6, n = 7; Figure 3.3 a). Approximately 10% of the larvae released into tented replicates were recovered after 24 hours. In contrast, only 0.29% of released larvae were recovered from untented crates.

Although there was a major decline in the numbers of survivors between 24 and 48 hours within the tented containers ($P < 0.001$, $F_{1, 16} = 17.167$), there was no significant decrease over time within the untented containers ($P = 0.326$, $F_{1, 12} = 1.048$; Table 3.3, Figure 3.3.b). Overall, higher numbers of larvae survived to 48 hours in tented crates (mean = 365) than untented crates (mean = 43; $P = 0.026$). Many more larvae settled under tents at Day 1, and even though a relatively large percentage of them did not survive to Day 2, initial densities were great enough such that survivorship at Day 2 was still an order of magnitude higher than in untented containers (Figure 3.3c). To examine the relative proportion of settled larvae that survived to 2 days, I used the ratio (Day1 - Day2) / Day1. This analysis showed that there were overnight losses of 85% in tented crates, and losses of only 40% in untented crates. The proportion of larvae surviving to Day 2 in untented crates was significantly higher than in tented crates ($P < 0.001$, $F_{1,14} = 21.427$).

3.4. Seasonal Differences in Settlement and Recruitment

Limited sampling (n = 2) indicated comparatively low initial settlement in Spring samples; only 0.52% of released larvae were found after 24 hours. In the Fall, initial settlement rates were extremely variable and spanned three orders of magnitude, ranging from 0.4% to nearly 40% of released larvae (Fig. 3.4.a, 3.4d, Table 3.4).

During the Spring settlement experiment, sampling seven days after larval release revealed that 0.58% of released larvae settled and metamorphosed (slightly higher settlement than at Day 1 reflected variation between cobbles). A month after seeding, only 0.04% of released larvae were found, equaling 6.9% of those surviving to one week. There was a significant decline in survivorship over time ($P < 0.001$, $F_{1,13} = 45.465$; Fig. 3.4.b). Surveys calculated 15 weeks after seeding found the equivalent of 20 abalone per m^2 of habitat searched. A final survey at 20 weeks yielded 28 abalone per m^2 habitat searched.

During the Fall experiment, surveys conducted at seven days found 2.37% of released larvae. Due to the extremely variable initial settlement at Day 1, declines in numbers over time were not significant. However, by one month, just 0.15% of released abalone were found.

Final surveys of the experimental crates took place in July and August of 1997, 10 months after the Fall 1996 larval release. Although 12 out of 17 crates were missing, the release areas where the crates had been tethered and any remaining cobble were searched carefully for juveniles. Ten juveniles were found in a total of

1.8 m^2 area (17 crates x 0.11m^2 per crate), equivalent to 5.3 abalone per m^2. Juveniles ranged in size from 12 to 35mm.

There was no difference between seasons in size of post-larvae at Day 1. Abalone grew significantly less during the colder Spring months (Fig. 3.4.d). Growth rates one week after settlement were 17.2 microns per day for those larvae released in the Spring, and 26.6 microns per day for those released in the Fall. After one week, Fall abalone were significantly larger, averaging 0.49 cm compared to 0.40 cm ($P < 0.0001$, $F_{1,191} = 1197.95$). Growth rates between 1 and 4 weeks averaged 8.2 microns a day in spring, and nearly double in the Fall, at 15 microns a day. After a month, Fall abalone were significantly larger than their same-age Spring counterparts, averaging 0.801cm compared to 0.580 cm ($P < 0.0001$, $F_{1,146} = 185.961$).

Discussion

Abalone larvae depend upon ocean currents for dispersal to and within settling grounds (Tegner and Butler 1985). Water motion over rocky reefs and kelp beds may not be as strong or directional as that over more open areas, and may act not only to trap but also to retain unattached larvae and thus facilitate eventual settlement (Eckman *et al*. 1989). However, extensive dispersal often occurs before larvae are competent to select a substrate and settle, so that many suitable settling sites may be passed (Palmer and Strathman 1981). While larval dispersal may be an important process for natural distribution of propagules, it can complicate any manual reseeding, by spreading the larval seed so thin that settlement success is difficult to

quantify.

Widespread abalone recruitment along the mainland did not exist by the mid-1990's possibly because adults, although of breeding size, were typically isolated from other individuals. However, even under intense fishing pressure, recruitment of red abalone was still occurring at San Miguel Island, as there remained a substantial population (just under the commercial harvesting size limit) in many areas around the island. The island's distance from the mainland and often inclement weather has prevented harvesting to the same degree that other islands and the mainland have experienced for the past century. I performed surveys in several areas around San Miguel to quantify natural recruitment as a standard for my larval release experiments. At Wykoff Ledge, three cohorts were evident. I suspect that the smallest class was between one and two months old, from a spawn in the warm water month of September. Since variation in size among individuals increases as abalone grow, I cannot be sure that the two larger size classes resulted from two separate spawning events, but it seems plausible. Natural densities of juvenile abalone appear to vary considerably in both time and space. Surveys of 'nursery rocks' reported in Morse *et al*. (1980) found densities of up to 224 juvenile abalone per m^2 of bottom surface area. My survey of recruitment on Wykoff Ledge at San Miguel Island revealed densities ranging from 0 to 28 per m^2, of which over 75% of individuals were between 1-4 mm (1-2 months post-settlement).

I found that sampling only a single cobble from each crate was obviously not sufficient due to the large amount of variability between replicate crates that masked

potential trends. As a result, I was not able to show that a high release density provided more survivors than the medium density treatment (Fig. 3.2b), although both of these treatments produced more survivors than the 'low density' treatment. Extremely high densities of potential settlers could have led to strong density-dependence and thus reduced recruitment success. From an economic perspective, there would be no point in transplanting high densities of larvae if releasing fewer larvae produced comparable levels of settlement. Additionally, the fact that two survivors were found after 12 days on a low release density replicate suggests the need to sample more area, not necessarily release more larvae. The lone larva found on a control cobble could potentially have been released at a nearby (4 m distant) high density treatment and been transported by currents to the control crate.

It is difficult to find newly-settled abalone on a cobble under a dissecting scope due to their small size and the cobble micro-structure. Settlement was probably underestimated due to the visually cryptic nature of post-larvae. Another technique, such as anesthesia (Prince and Ford 1985) or suction sampling (McShane and Smith 1988), would be a preferable method because it would facilitate collection and quantification of newly-settled larvae.

Tented crates clearly allowed for greater settlement, probably by retaining the larvae within the crate until they had a chance to settle. This led to overall an order of magnitude higher density at 48 hours. However, proportionally, mortality was also much greater. The number of survivors in tented crates at 48 hours was only 14% of initial settlement, while in the untented crates, the low initial densities of settlers did

not decline significantly.

Tenting was shown to be an effective mechanism for increasing the numbers of initial settlers, and thus leading to overall higher densities after 48 hours. It is likely that the higher mortality in tented replicates was related to some sort of density-dependent effect such as increased stress, or susceptibility to predation. Settlement densities at 24 hours in the tented crates were approximately 4-5 times greater than in the untented crates. It is possible that weaker larvae were able to settle under tents in the absence of water motion, but once the tents were removed, they were unable to remain attached to the substrate. Ebert and Houk (1984) report that almost half of initial mortality consists of veligers that do not survive past metamorphosis. Photographs and scanning electron microscopy of the shells of the post-larvae at 48 hours reveals new shell growth (Fig. 3.4.e, 3.4.f), which is evidence of successful metamorphosis. Additionally, as survivorship from planktonic larvae to benthic juveniles can be as low as 10% under constant laboratory conditions (Hahn 1989, Ebert and Houk 1984), the chance of finding any settlers under field conditions becomes slim.

I observed settlement densities that compare favorably to estimates of natural abalone settlement. Keesing *et al.* (1995) report settlement peaks of 114 per m^2 and 45 per m^2 for *Haliotis rubra* and *H. laevigata*, respectively. Nash *et al*. (1995) recorded peak settlement of *H. rubra* equivalent to 2347 larvae per m^2. After 48 hours, my tented crates averaged over 3000 settlers per m^2, while the untented crates averaged 390 per m^2. Although relative survivorship in abalone that settled under

tents may be lower than that of those that were released into open water, the tents clearly are a mechanism for promoting dense initial settlement. How do these numbers compare with other work? For *H. ruber*, Garland *et al*. (1985) reported seeding tanks with densities of 1 larva per ml. Mean survivor density was 9000 per m^2 (0.9 per cm^2) after 6 weeks and 6000 per m^2 (0.6/cm^2) after 13 weeks. Under closely controlled laboratory conditions, Ebert and Houk (1984) averaged 7.5% survivorship at 3 months.

Several factors could be responsible for lowered settlement and survivorship in the Spring. Rough conditions led to strong surge, and elevated amounts of suspended particulates, which could have negatively impacted post-larval survivorship. However, since all of the crates were tented for the first 24 hours, I expected that initial impacts would be minimized. Observations showed that the strong water motion caused the larval retainment bags to sway and billow from side to side, and it is possible that this movement prevented some larval settlement. A more likely explanation, however, was the extremely turbid conditions. Water visibility fell from 5m to less than 1m on the day of release, and it is possible that as sand and particulates settled out of the water, larvae were impacted by physical abrasion. Numerous field observations suggest that settlement and survivorship are diminished on silty substrate, both for abalone (pers. obs.) and for other species (Gotelli 1988). A high-energy ground swell occurred during the first week of the Spring experiment (pers. obs). Such swell, combined with local wind and currents, provided unfavorable conditions for larval survival. Although work on Japanese

abalone has indicated that spawning events are triggered by high wave activity, such as during typhoons (Sasaki and Shepherd 1995), other observations for Australian (Prince *et al*. 1988), New Zealand (McShane *et al*. 1988), and Eastern Pacific (Breen and Adkins 1980) species indicates that spawning events occur during calm periods, or when a mild reversal of normal current patterns may help to retain spawn within the natal reef. During the Fall experiment, calm conditions existed within the Santa Barbara Channel and higher settlement, survivorship and growth rates occurred. Spring water was on average 5° centigrade colder than Fall water (pers. obs.) and it is possible that the warmer Fall water fostered higher growth rates. Even though Fall survivorship dropped by an order of magnitude over the first week, initial settlement was higher than in the spring.

The abalone recovered from the experiments were either intact or were empty shells. Empty shells could indicate mortality from causes other than predation, such as starvation or poor water quality (Gosselin and Qian 1997). However, as intact individuals within samples appeared uniformly robust, often with full guts, I decided to attribute empty shells to predation. Predation upon larger individuals is often evident because the shells are chipped or damaged. However, since shells of month-old or younger abalone are fairly fragile, and any shell damage might have been from handling rather than due to predators, I did not quantify damaged shells. I assumed that empty shells had been preyed upon shortly before sampling, as empty shells may quickly reabsorb in an ocean environment (Hahn, 1989). Empty shells significantly smaller (with less growth) than the majority of recovered individuals might indicate

mortality at a younger age. However, I did not recover any small larval shells on any day than Day 1. Additionally, all empty shells showed some growth. It is possible that empty shells are indicative of a mass predation event, rather than be due to local abiotic conditions which would affect all crates equally. The range of empty shells recovered indicates that predators were present in some crates and not others. For example, crate 17 in the Fall had 105 intact abalone and no empty shells, while nearby crate 15 had 288 empty shells and only 38 intact abalone. A single small octopus can easily eat a dozen 30mm abalone in a matter of minutes (pers. obs.). Overall, mortality appeared higher during the Spring experiment.

Red abalone have been induced to spawn in the laboratory every month of the year if food is not limiting. What determines the best time for natural spawning or for larval settlement and survivorship, and are the two processes tightly linked? Some anecdotal evidence suggests that changes in water temperature (Breen and Adkins 1980) or motion (Sasaki and Shepherd 1995) can induce spawning, and such physical cues may be critical to insure larval survival. Under field conditions, it has been found that the spawning season for red abalone runs mainly from April through July, but gravid animals may be found in the fall (Cox 1962) and sometimes year-round (Young and DeMartini 1970). Evidence from the laboratory and field has shown that both growth and gonad production are linked to adult food supply (Ebert and Houk 1984, Tutschulte and Connell 1988). Although metabolism (and thereby growth rates) increases with warmer water, algal food tends to decrease. Conversely, while colder water provides nutrients fostering algal growth, abalone metabolic rates are

slower. Therefore, optimum spawning should occur at some time following new growth of kelp due to spring upwelling of cold, nutrient-rich water.

What are the ecological implications of the strong differences in growth linked to water temperature? Faster growth means a shorter time spent in vulnerable size classes. Optimal temperatures for growth of juvenile red abalone range between 15 and 21°C (Leighton 1974). Above these temperatures, algal food declines and water oxygen levels may fall. Growth of juveniles even under constant lab conditions is extremely heterogeneous (pers. obs. and as reviewed by Hahn 1989), so extrapolating age from size in wild individuals may be impossible. However, I knew the age of my outplanted larvae, and so was able to estimate growth rates over two time periods: 1-7 days, and 7-28 days after settlement. Even though there was some variation, larvae grew significantly larger, faster, in the 17° C Fall water than in the 12° C Spring water. Daily shell growth rates as shown in Hahn (1989) for juvenile red abalone when tested from 12 to 24° C are highest at 18° C, although the initial size of the animals tested was not given. There remains the possibility that my estimates of Spring growth rates during the first 7 days are inaccurate, as 99% of the shells recovered were empty. Predation might have happened several days prior to sampling, resulting in smaller, younger shells in the sample. However, as Spring shells were still significantly smaller than Fall shells at 28 days, when only 36% of Spring shells were empty, I conclude that spring water temperature may have been a contributing factor in lower growth.

Chapter Four. Benthic Species as Indicators of Suitable Settlement Conditions for Red Abalone

Introduction

The role benthic invertebrates play in structuring their environment has been examined by many ecologists (Duggins 1983, Dayton 1971, Sousa 1979). As well as benefiting the individual, such changes may affect other species. For example, abundant herbivores, such as urchins, may control the extent and/or condition of 'urchin barrens'-- areas of coralline crusts free from most other foliose microalage (Cameron and Schroeter 1980). These barren areas, common near habitats occupied by adult abalone, may be important for abalone larvae, as crustose coralline algae contain the chemical cues needed to induce settlement (Morse and Morse 1984). While urchins or other grazers maintain such substrates, they may also impact settlement. Although urchins may prey upon some post-larval species, there is evidence that such 'predation' might actually result from either bulldozing or other physical interference (Fletcher 1987, Maldonado and Uriz 1998). In addition to urchins, grazers such as the limpet *Lottia* sp. have been shown to "bulldoze" and physically remove post-larvae from the substrate and thus affect settlement of other species within their territories. Therefore, while the presence of some species may create good settlement substrate, their activities may physically prevent settlement from occurring.

Urchins may also provide shelter for juvenile abalone from predation under

their spine canopy, as shown for spiny lobsters by Davis (1971). This could be of importance for red abalone and the coexisting red urchin *Strongylocentrotus franciscanus*. As the urchin fishery continues to grow, increased fishing effort is occurring at San Miguel Island, the last Southern California stronghold for red abalone. Tegner and Dayton (1977) and others have shown both post-larval urchins and abalone are sheltered beneath the spine canopy of adult urchins on barren grounds. Large, reproductive urchins are selectively fished, thereby not only removing both urchin broodstock and nursery ground for larvae of several species, but also impacting the process that keeps coralline crusts 'clean' (shown to induce settlement in Chapter 2). Crusts that are no longer grazed by urchins may become overgrown with diatom films, and their inductive surfaces may no longer be available for exploring larvae. Ebert and Houk (1984) have shown that if the diatom layer is too thick, abalone larvae may become tangled and never actually reach the coralline surface to begin metamorphosis.

Rather than selecting urchin barrens as generally good locations for abalone larval seeding, it may be important to examine areas on a finer scale for other processes that could potentially significantly increase or decrease larval settlement and survivorship (Gosselin and Chia 1995). In addition to species that maintain substrate condition, it is possible that other newly-settled invertebrates could as act as indicators of 'good' settlement conditions. Small benthic bivalves and mollusks are common within nearshore kelpbeds along the Santa Barbara coast. Although adults of common species as *Pododesmus cepio* and *Parapholus* sp. are easy to identify, not

much is known about their recruitment patterns on either a spatial or temporal scale. However, factors that affect abalone larval settlement and survivorship could also affect similarly-sized larvae of these and other local species. Choosing habitat that supports small gastropods and mollusks as a release site for abalone larvae could increase chances for successful settlement.

My studies presented in Chapter 3 using natural coralline substrate and seeded abalone larvae provided an opportunity to examine the concurrent settlement, growth and seasonality of wild mollusk recruits, and the resulting information is presented here. Additionally, I decided to use natural recruitment of locally occurring species as a proxy of 'good' conditions by which to gauge potential success of abalone outplants. Knowledge of successful wild recruitment of other species could prove useful for finding 'good' areas for future abalone seeding.

In the following sections I describe an experiment that examines the role urchins may play, both directly and indirectly, in abalone settlement success. The results of field surveys for new abalone recruits at San Miguel Island are described, and then I explore the possibility of using other newly-settled invertebrates as a proxy for good abalone habitat.

Methods

4.1 Role of Urchins

Trials took place in flowing seawater tanks at the Marine Science Institute, UCSB. I chose for the experimental substrate sanded Plexiglass plates (10cm by

10cm) that had been cultured in a shaded flowing seawater tank for one year, during which time they had become covered with the encrusting coralline algae *Lithophillum* sp. To test larval settlement success as a function of substrate condition, I prepared two 'film' conditions ('algal film', 'no algal film') crossed with two 'grazer' treatments in the following manner. I placed plates into separate Tupperware containers. To create 'unfilmed' conditions, six plates were incubated in a shaded flowing seawater tank. Shaded conditions prevent overgrowth of diatom films. The remaining twenty containers were placed into an uncovered tank. The higher light level in this tank allowed for diatom growth, and these plates are referred to as 'filmed'. All containers and plates were left for a week. After a week, I placed one small (test diameter < 40mm) purple urchin into each of thirteen 'filmed' containers. Containers were covered with coarse nytex mesh to keep urchins from escaping. Urchins were allowed to graze the plates for two days. I then removed urchins from six containers, and left them in the remaining seven containers. The resulting four treatments were unfilmed + ungrazed (n = 6), filmed + ungrazed (n = 7), filmed + grazed + urchins removed (n = 6) and filmed + grazed + urchins present (n = 6). All containers were then placed on a table and flushed with fresh seawater. Flow was stopped so that the water level in each container was just below the top. Abalone larvae were a week past the onset of competency and were obtained from The Cultured Abalone, a commercial mariculture facility in Ellwood, CA. Approximately twenty larvae were pipetted into each container. Unfortunately, three replicate containers from the filmed + ungrazed and one container from the filmed + grazed +

urchins removed treatments were lost and were not scored. After two days each plate was carefully removed and examined under a dissecting microscope, and all abalone were counted. I discussed the results of unfilmed + ungrazed and filmed + ungrazed in Chapter 2. I used one-way ANOVA to determine differences between treatments.

4.2 Natural Recruitment: Density and Microhabitat Choice

To quantify density and microhabitat choice of wild abalone recruits, I sampled reefs on both sides of San Miguel Island. In late September 1996 at Nifty Rock (on the north side of San Miguel Island) near a healthy adult red abalone population in Nifty Cove, I searched haphazardly for 30 minutes along a vertical wall both in crevices and beneath urchins for recruits (Figure 4.2a). In November 1997 at Wykoff Ledge (on the south side of the island), a historically 'good' area for abalone fishing (John Colgate, pers. comm.), I searched 13 random 0.25 m^2 quadrats for both urchins and abalone recruits.

4.3 Gastropod and Bivalve Recruits

Collection of naturally-occurring gastropod and bivalve recruits took place concurrently with the seasonal abalone seeding experiments presented and discussed in Chapter 3. Handling of experimental units and samples was described in detail in Chapter 3. All cobble rock provided as settlement surface was handled and brushed lightly before placement in the crates, to remove any newly-settled organisms. Because of this, all individuals recovered were assumed to have recruited during the

course of the experiment. Preparation of crates took place during the two days before crates were bagged and abalone larvae were released. Cobble from crates was collected at 1, 7 and 28 days after seeding with abalone larvae.

For both Spring and Fall experiments, recent gastropod and bivalve post-larvae less than 1mm were sorted from the samples, preserved, counted, and measured. Recruits were scored into apparent functional groups based on shape and on the surrounding adult community, as it was very difficult to identify to species. The most common of the gastropods was referred to as Snail 1 (a small brown *Tegula*-shaped snail). The remainder ('other snails') were either various small, recent post-larvae, only distinguishable from one another by protoconch shell sculpture and by shape of new growth, or other microsnails.

The 'bivalve' category consisted mainly of three types: Clam 1 (all typical clam-shaped recruits), Clam 2 (elongated pholad shape) and Oyster (a flattened *Pododesmus*/scallop-shaped juvenile). Clam 1 and Clam 2 from all samples were measured. Other bivalves and gastropods were not common enough to analyze. For simplicity of comparison in this discussion, all recruits were grouped into either 'gastropods' or 'bivalves'.

Recruitment densities and size classes of gastropods and bivalves were analyzed in both Spring and Fall, compared to each other and to seeded abalone survivorship. Pairwise correlations were run. For clarity, in this section 'other recruits' will be used to describe all of the naturally-recruited post-larvae.

Results

4.1. Role of Urchin Grazing

The treatments tested the settlement success of larvae on filmed and grazed plates, in the presence or absence of urchins. Larval settlement appeared to be dependant upon substrate condition (Figure 4.1), as only 2.5% of larvae settled in the presence of an algal film. In containers where the film was removed by urchin grazing, but the urchins remained in the containers, settlement was not significantly higher. However, settlement increased significantly if the film was first removed by urchin grazing, and then the urchins removed before the introduction of larvae ($P = 0.001$; $F_{1,7} = 26.429$). There was no significant difference in settlement between plates with the grazers removed and those plates that had been cultured under low light conditions and so were never filmed initially.

4.2. Natural Recruitment: Density and Microhabitat Choice

Sampling for juvenile abalone, although limited in scope, proved successful in finding varied sizes of individuals (as pictured in Figure 4.2.a).

The results of searches for abalone recruits in a variety of habitats are shown in Figures 4.2b-d. There were roughly six times as many juvenile urchins as there were juvenile abalone in random quadrats at Wykoff Ledge (Figure 4.2.b). More abalone were found under red urchins than in other microhabitats at Nifty Rock (Figure 4.2.c). These recruits tended to be smaller than those individuals found either in cracks, under seastars, or in the open (Figure 4.2.d). Recruits beneath urchins were

significantly smaller (mean = 12mm) than those in crevices (mean = 18mm; P = 0.0047, $F_{1,25} = 9.6537$).

4.3 Gastropod and Bivalve Recruits

The abundance of bivalve and gastropod recruits are compared with abalone abundances in Table 4.3. Numbers are shown for each individual experimental plot (area= $0.11m^2$), for all time periods. Bivalves were more common than gastropods during the Spring, by almost an order of magnitude. In the Fall, far fewer bivalves were found within plots while the numbers of gastropods generally did not differ between seasons, although there was one crate that contained a very large number (1307 out of a total of 1309) of small snails (Table 4.3.1).

Abundance of gastropods and bivalves were positively correlated in the Spring experiment ($r^2 = 0.737$, $P < 0.001$, Figure 4.3.2). There was no relationship between recruitment of gastropods and bivalves during the Fall ($r^2 = 0.08$); far fewer bivalves were found within plots while gastropods did not change significantly (Table 4.3).

I also compared the densities of naturally-occurring gastropods and bivalves with the density of seeded abalone. As more samples were collected at Day 28 than other times, and since I was interested in comparing abalone recruit densities, I present only those results here.

During the Spring experiment, plots with higher numbers of abalone tended to have higher densities of other gastropods and bivalves (Table 4.3, Figure 4.3.3). The

density of abalone surviving to 28 days was correlated with the total number of bivalves (r = + 0.75, P = 0.019) and total gastropods (r = + 0.91, P < 0.001).

During the Fall, abalone were positively correlated with bivalves (r = + 0.88, P = 0.021) but were not correlated with gastropods (P = 0 .39). There was no significant difference between density of Day 28 seeded abalone and total gastropod recruits in either the Spring or the Fall samples. However, bivalve density was significantly higher than abalone density in the Spring (single factor ANOVA, P < 0.001, $F_{1, 16} = 16.46$).

Discussion

This chapter explores the ways in which other organisms could be indicators for red abalone settlement and survival success. As mentioned earlier, knowing what to look for, such as the presence of red urchins or newly-settled gastropods, may increase the chances for settlement success of abalone and maximize return for the effort. Some aspects of the habitat may be common requirements for settlement shared among many invertebrates, and by identifying these and outplanting in areas where they are maximized, better recruitment of outplanted abalone larvae may result. Factors could include the presence of another organism (sheltering by urchins or adult abalone), the condition of the settlement surface maintained by another organism (coralline crusts grazed by urchins), or the presence of newly-settled larvae (abalone or otherwise) indicating an absence of predators.

As expected, the presence of a thick diatom film discouraged larval

settlement. If the diatom layer is too thick, larvae may become tangled and never actually reach the coralline surface to begin metamorphosis (Ebert and Houk 1984). Eventually, some of the larvae did settle on the edges of the plates where the diatom film was thinner. And as also expected, urchins can mediate settlement by grazing the diatom films. A grazed film, with the urchins then removed, was just as attractive a substrate as a clean coralline crust which had been cultured under low light conditions. Roughly half of the available larvae settled and survived under these conditions. Conversely, the presence of an urchin interfered with settlement, resulting in much lower success (< 20%). Maldonado and Uriz (1998) found that the utilization of micro-refuges by post-settler sponges reduced mortality from bulldozing urchins. In my experiments, the urchins, although relatively small, actively scoured the plates, occasionally removing crusts and possibly settled larvae as well. Hence, there was little spatial refuge from the grazing urchins, as the flat Plexiglass plates lacked rugosity common in natural substrate. Under field conditions, Fletcher (1987) showed that grazers, especially urchins, appeared to be necessary for maintenance of crustose algae. Unfortunately, the urchins overgrazed my flat plates to the extent that all crust was removed. Creese and Underwood (1982) have shown that irregularity of the substrate may reduce grazing (and therefore bulldozing) efficiency in limpets. Rocky reefs are often pitted and bumpy on the scale of an Aristotle's Lantern or larva (per. obs.), and this may enhance post-larval survivorship.

The filmed + ungrazed plates appeared to be heavily overgrown with a reddish brown diatom film. Almost all of settlement on these plates occurred on the sides of

the plates, which were not as overgrown. Larvae may be unable to penetrate a film thicker than a few microns, becoming trapped and unable to undergo metamorphosis (Mathews and Cook 1995). My data further implicate the importance of grazers, such as urchins, in removing thick films from settlement surfaces. Additionally, the results suggest that artificial substrates, such as flat Plexiglass plates, are not the best experimental surfaces, and that natural substrates, such as cobble rock, might provide more small-scale habitat heterogeneity. It would be interesting to run this experiment again, using natural rock instead of Plexiglass plates, to examine the degree to which fine-scale rugosity provides a spatial refuge.

Clearly, it is important to have both grazer activity and a spatial refuge from bulldozing on the settlement surface. In natural systems, the amount and efficiency of grazing would depend on local grazer densities as well as rock type and swell exposure. On exposed reefs, as at the north and west side of San Miguel Island, water motion prevents many urchins from active grazing outside of their home scars. Sedentary urchins provide not only patches of 'clean' substrate, but also spatial refuge beneath their spine canopy. In calmer areas, or with less drift algae, urchins are much more mobile, and they may contribute less towards settlement and survivorship of abalone.

My surveys of recruitment at San Miguel reinforce the idea that abalone recruits co-occur with urchins. Although I did not directly test the hypothesis that recruits utilize urchins as a spatial refuge, qualitative field observations suggest that shallow, more turbulent habitat aids in transport of drift kelp and prevents urchin

movement, and thus favors congregation of abalone beneath the spine canopy. In deeper areas where there is less water motion (meaning less drift and less disturbance) urchins roam from their home scars, leaving abalone recruits exposed to potential predation. All of the small abalone observed were beneath urchins, and were more common in shallower, more turbulent areas. I did not find any new recruits at Nifty Rock in early fall. Judging by size classes, the smallest abalone found was between 4 and 6 months old, suggesting that they recruited from a spawning event in the previous spring. A separate, slightly larger group found refuge in crevices.

I compared natural recruit density to the density of month-old seeded abalone (Chapter 3). I had to assume that natural densities of abalone recruits might be similar to those of other recruits if basic conditions such as risk of predation, habitat quality, and food sources were shared. The abundance and distribution of bivalve and gastropod post-larvae suggest that bivalves tended to recruit more heavily in the spring, while gastropods may recruit at lower numbers over a longer season. The crates that contained the fewest abalone also had the fewest bivalves and gastropods. Good conditions for abalone appeared to also be good for other mollusks. This suggests that a good method for choosing potential re-seeding areas might be to first examine the substrate for the presence of other wild species.

It is reasonable to assume that benthic gastropods with similar reproductive strategies might have similar patterns of larval dispersal and settlement, from a purely mechanical basis. As I was unable to culture any of the recovered recruits, I cannot

be sure of the species or life history characteristics. However, my data suggest that some mechanisms, such as predation, may affect all young recruits regardless of reproductive strategy. The high correlation of gastropod density with abalone density in the Spring Day 28 samples suggests that many of the same processes are important on an inter-species level. Obviously, more work needs to be done to target appropriate proxy species for abalone, and this will depend on the area that is to be seeded. Initial surveys for species abundance and distribution should be performed. The results suggest that 'good' settlement habitat can be inferred from examining naturally-occurring recruitment on local substrates, and I recommend that this should be done before any abalone larval seeding take place.

Chapter Five. Outlook for Outplanting

The suitability for larval outplanting as a means of augmenting populations of red abalone depends in part on the definition of 'restoration' and 'enhancement'. These words can denote different things depending on whether it is an ecological or fishery perspective. A population ecologist would be interested in bringing stocks of a depleted species up to a level at which they can be sustained. From a fisheries perspective, however, the species must not only be able to maintain its population numbers, it must be able to withstand some degree of fishing pressure as well. Whether or not these levels can be the same remains up for discussion. It may not possible for abalone to withstand intense harvesting for more than a few years without eventual population collapse. Previous fishery management was based upon deterministic models which did not account for wide variation in settlement success (Tegner *et al*. 1989). All California abalone fisheries eventually showed classic signs of serial depletion (Tegner *et al*. 1989). Other countries, such as Australia, have implemented harvesting regulations that may spare local populations from continuous pressure. A stock recruitment model for *H. laevigata* indicates that once adult populations drop below a critical level, recruitment failure and population collapse is likely (Shepherd and Partington 1995). A stock reduction analysis of the Mexican fishery suggested that populations are heavily exploited and are being serially depleted, and that abalone fisheries in general are unproductive by nature (Prince and Guzman del Proo 1993). Red abalone is a species that has been intensively harvested

commercially for several decades and harvested by native interests for perhaps thousands of years. Although populations in Southern California experienced a historic release from intense predation by the California sea otter, hunted to near extinction in the late 1800's, otters are currently moving south of Point Conception. Foraging otters have heavily preyed upon remaining abalone (commercial fishermen, pers. comm.). Stocks already depressed from harvesting and increasing otter predation also potentially face the infectious disease Withering Syndrome (WS), which reduced intertidal black abalone (*H. cracherodii*) populations by up to 98% (Altstatt *et al*. 1996). Indeed, DFG biologists found that up to 5% of red abalone encountered showed signs of WS (Pete Haaker, pers. comm.). Another looming threat is the sabellid shell worm that currently infests all abalone hatcheries in California (Culver *et al.* 1997). Fear that this South African parasite could potentially spread through wild populations forced DFG to pass a moratorium on outplanting hatchery abalone of all sizes, including larvae, in 1997.

It will be difficult to determine what density of abalone is 'natural', as we have harvested them constantly throughout the historical record. Additionally, abalone are only part of a whole community that has been subject to increasing fishing pressure, and we may never know the ramifications of alteration of densities of major co-occurring predators such as crabs, lobster and various fin fish.

Fishery *restoration* demands cost-effective methodology, as the goal is to produce the greatest number of harvestable abalone in the shortest time frame. Additionally, the goal may be to restore the fishery to some historic catch statistic.

Fishery *enhancement* suggests merely augmenting the number of fishable adults. Population *restoration* denotes increasing a local population to some level at which it functions at a reproductive level. Population *enhancement* suggests increasing the stocks within a local area, with the intent of eventually establishing a breeding population. Given this definition, any increase in density is enhancement, especially within areas such as my study site, where there were no abalone prior to my experiments.

My research found that post-larval survivorship can vary widely, not only on time scales from seasons to years, but on very small spatial scales as well. This variation is entirely natural and especially inherent in long-lived, fecund marine invertebrates. Laboratory culture has shown that mortality during the first month can approach 90%, and most of this occurs during the initial 2 weeks after settlement (Ebert and Houk 1984). Even in the predator-free high-food environment of culture tanks, survivorship averaged 7.5% during the first three months (Ebert and Houk 1984).

For economic considerations, whether larval outplanting can be cost-effective will be based in part on the cost of larvae. Some of the larvae I used were produced in labs at UCSB, while others were purchased from a local hatchery. Obviously, hatcheries are concerned with making a profit, and the cost of the larvae may depend upon factors including the size and sale of the operation. For my last experiments, I purchased 1 million hatchery larvae for $300. Cost per survivor, after a week, ranged from $0.03 to $0.08 in the Spring, and from under a penny up to $0.20 in the Fall. A

month after outplanting, juveniles 'cost' as little as $0.04 each, but in some cases the entire experiment failed and there were no survivors. My experiments did not assess emigration of juveniles out of the area, and so my data most likely under-represent actual survivorship.

It is apparent that minimizing initial costs of the larvae is crucial if outplanting techniques are to be cost-effective. I believe that, even if there is occasional failure, outplanting larvae is still a viable option. Hatchery adults usually can be spawned whenever a batch of larvae is required, such as when physical conditions warrant release. Competent larvae may be delivered to a local area, and if settlement and survivorship appear to be low after a day or two, the outplant could be repeated. Obviously not every natural spawning event is successful, as wild abalone occur in distinct size cohorts perhaps many years apart. By outplanting larvae, our goal is to provide larvae that cannot otherwise be supplied from adult broodstock. Although there is concern about a potential decrease in genetic variability due to releasing farmed abalone, evidence suggests that short-lived lecithotrophic larvae may not effectively facilitate gene flow on any scale (Parsons 1996) and the loss of wild genetic variation can be offset by obtaining spawn from a large number of adults.

Larval settlement may be assessed even within a few hours after release, while larger juveniles (that escape immediate predation) retreat to cryptic habitat, thus becoming difficult and time consuming to find. Larger juveniles may require special treatment at release in order to prevent shock or stress that may lower survival. The less amount of time juveniles are held, the lower this stress may be.

My data found that individuals that survived to one month of age had up to a 40% chance of surviving to 5 months. I believe that my data show a worse-case scenario, as my experiments were plagued with both natural and man-made disturbances. Several unseasonable and unusual storm events swept through my site, causing major damage and losses of experimental containers, and increased scouring and silt, both shown to decrease survivorship in recently settled abalone. Additionally, lobster traps were frequently deployed and recovered in the immediate vicinity of my experiments, finally resulting in entanglement and losses of 80% of my experiments (Figure 5). Given the degree of unexpected disturbances, it was surprising that I could find any survivors at all. Therefore, I remain optimistic about the possibility of enhancing wild populations with outplanted larvae. The deciding factor will be if large supplies of larvae can be produced at minimal cost, and be available when field conditions are optimal for settlement. I feel that outplanting larvae is a viable means of *enhancing* wild populations, thus bringing about eventual *restoration*.

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Figure 1. Location of field experiments. Study site is at Isla Vista Reef, Santa Barbara County, CA (34° 24' 150" N, 119° 51' 620" W).

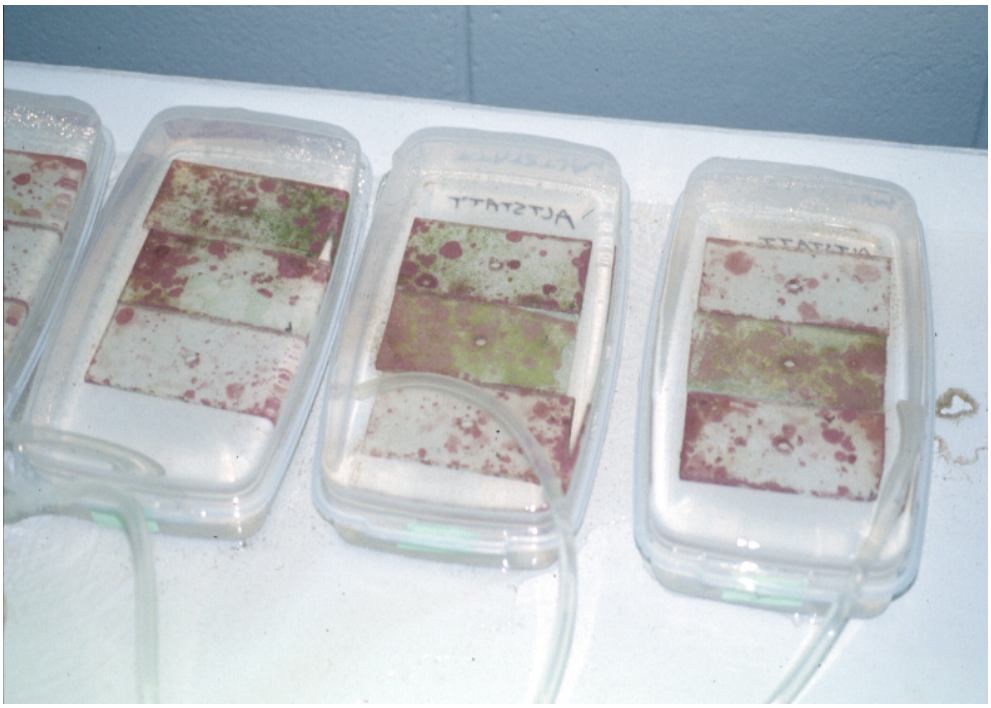


Figure 2.0. Photo of coralline-crust covered Plexiglass plates used in laboratory settlement experiments. Trays shown are approximately 15 cm by 65 cm. Most plates, and all microscope slides, had nearly 100% cover of coralline algae.

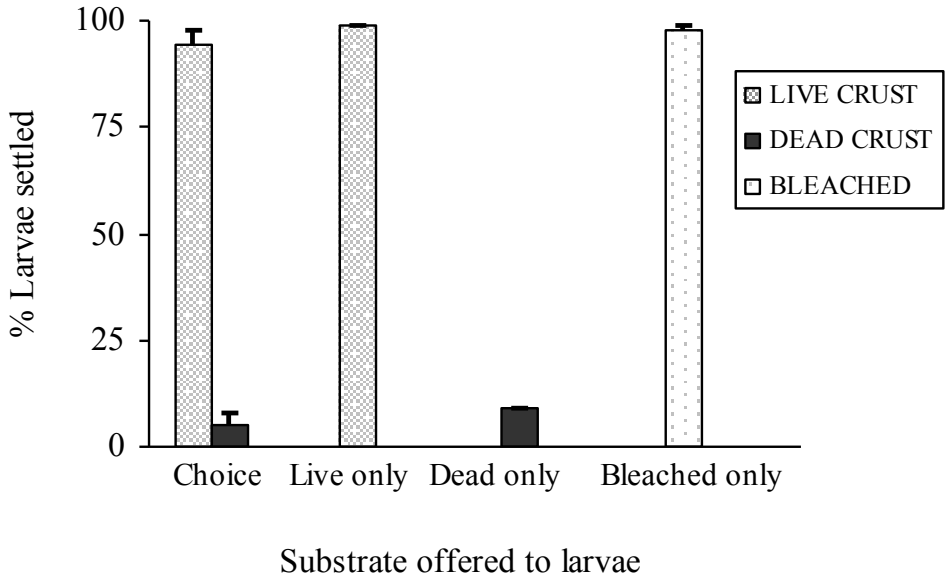


Figure 2.1.1. Data from preliminary substrate choice 24 hour trial. Results shown for larvae offered a choice of live and dead crusts (n = 2), live crusts only (n = 1), dead crust only (n = 1), or bleached crusts only (n = 2). Data are either means (+/- 1 SE) or totals.

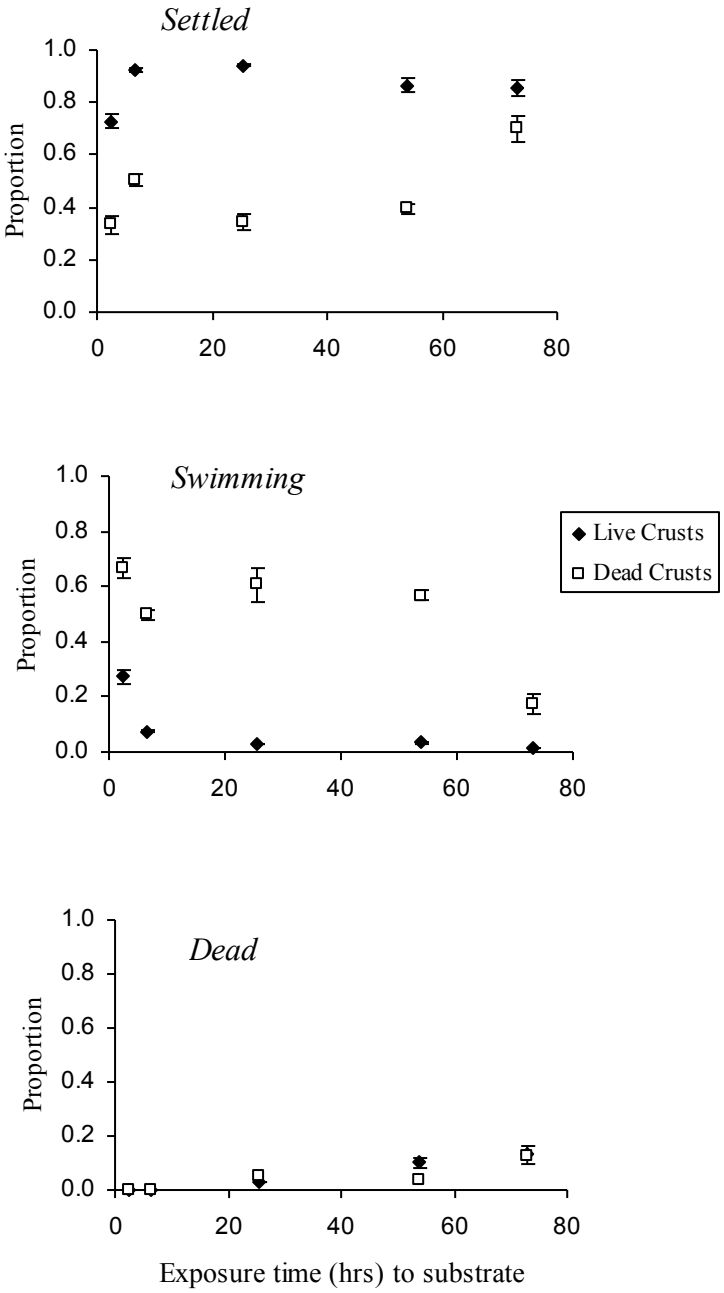


Figure 2.1.2. Proportion of larvae exhibiting different behavior (settled, swimming, dead) over time when exposed to either live or dead crusts (n = 9). Data are means (+/- 1 SE).

Table 2.1.2 ANOVA table for the effect of crust type on larval settlement. Larvae (approximately 20 per well) were exposed to either live or dead crusts over five time intervals. n = 9 for each time period.

Source of variation = Crust type		*df*	*MS*	*F*	*P*
Settled	2.5 hours	1	0.694	23.049	0.000
	Error	16	0.030		
	6.5 hours	1	0.797	57.039	0.000
	Error	16	0.014		
	25.5 hours	1	1.604	94.500	0.000
	Error	16	0.017		
	53.75 hours	1	1.005	46.676	0.000
	Error	16	0.022		
	73 hours	1	0.110	2.710	0.119
	Error	16	0.041		
Swimming	2.5 hours	1	0.694	23.049	0.000
	Error	16	0.030		
	6.5 hours	1	0.797	57.039	0.000
	Error	16	0.014		
	25.5 hours	1	1.499	47.774	0.000
	Error	16	0.031		
	53.75 hours	1	1.286	126.704	0.000
	Error	16	0.010		
	73 hours	1	0.115	6.319	0.023
	Error	16	0.018		

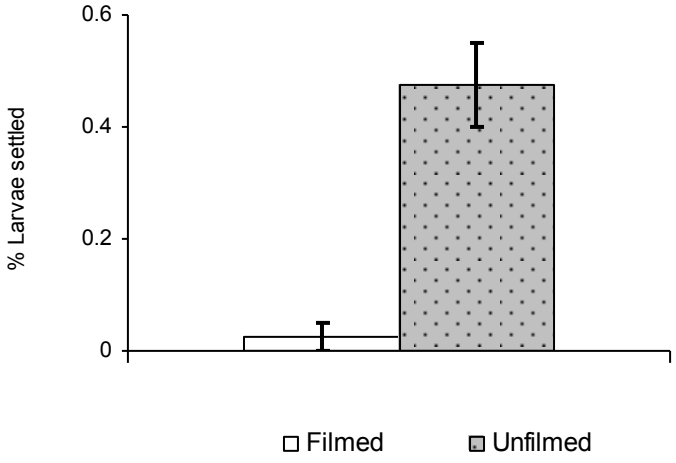


Figure 2.1.3 Effects of algal films on larval abalone settlement. Data are means (+/- 1 SE) for the percentage of abalone larvae settled over a 24 hour period on filmed (n = 4) or unfilmed (n = 6) crusts.

Table 2.1.3 ANOVA table for the effect of algal films on settlement surfaces on larval settlement (filmed n = 4, unfilmed n = 6).

ANOVA Single Factor

Source of Variation	*df*	*MS*	*F*	*P*
Substrate condition	1	194.4	22.70365	0.001418
Error	8	8.5625		

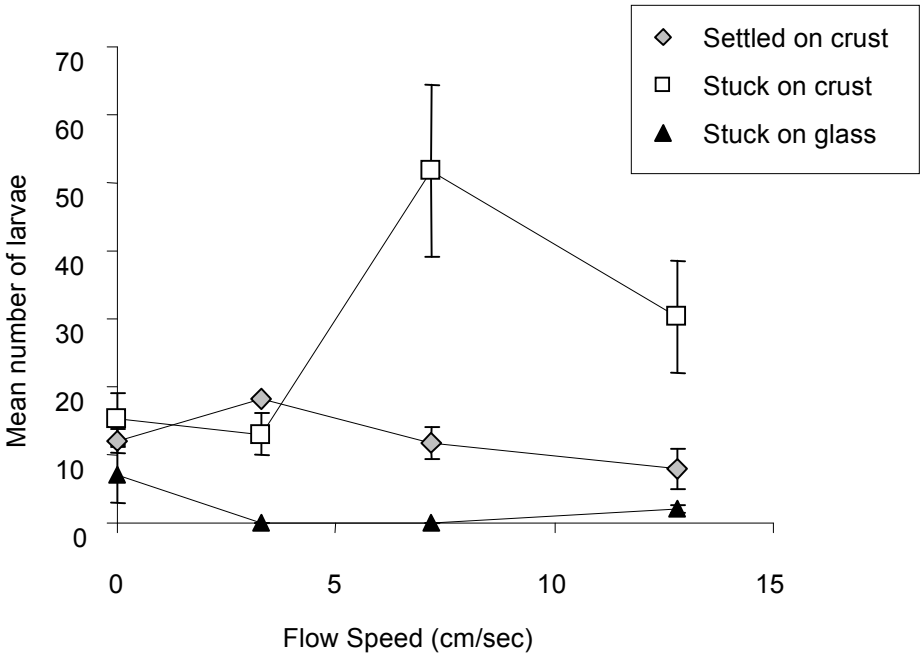


Figure 2.2.1. The effect of flow speed upon larval settlement. Larvae were assessed as either settled or stuck on either crust or glass slides. No larvae settled on glass slides. Data are means (n = 3, +/- 1 SE).

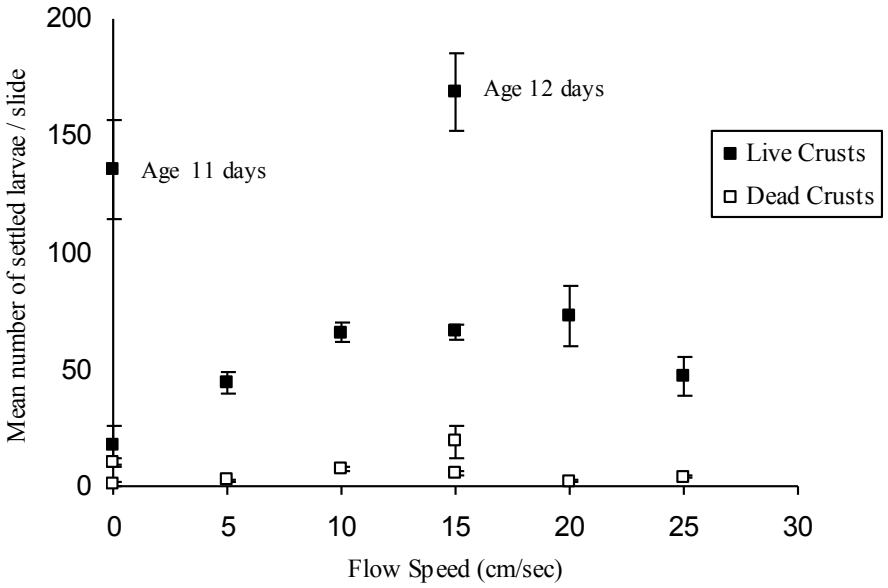


Figure 2.2.2. Number of larvae settled on live and dead crusts at different flow speeds. Data are means (+/- 1 SE) for each treatment (n = 3). Two outliers are oldest larvae used in experiment.

Table 2.2.1. ANOVA tables for the effects of flow speed on larval settlement. The four levels of flow were 0, 3.28, 7.16 and 12.8 cm/sec. Analysis is shown for larval behavior: settling on crusts, sticking to crusts, sticking to glass.

larval settlement on crusts	df	MS	F	P
FLOW	3	37.861	2.417	0.141
ERROR	8	15.667		

larvae sticking to crusts	df	MS	F	P
FLOW	3	950.306	4.982	0.031
ERROR	8	190.750		

larvae sticking to glass	df	MS	F	P
FLOW	3	38.000	2.868	0.104
ERROR	8	13.25		

Table 2.2.2. ANOVA table for the effect of flow speed and day on larval settlement on two different crust types (n=3). Trials ran for 24 hours. Six flow speeds were tested.

Analysis of Variance

Source	df	MS	F	P
SPEED	3	0.018	1.345	0.295
DAY	1	0.017	1.307	0.269
SPEED*DAY	3	0.009	0.716	0.557
ERROR	16	0.013		

Analysis of Variance without interaction factor

Settlement	df	MS	F	P
SPEED	5	0.022	1.688	0.191
DAY	1	0.009	0.737	0.403
ERROR	17	0.013		

Figure 3.0 Experimental crates filled with coralline crust-covered cobble rock. Rocks were collected from Isla Vista Reef. Approximate surface area of crate = 0.11m^2.

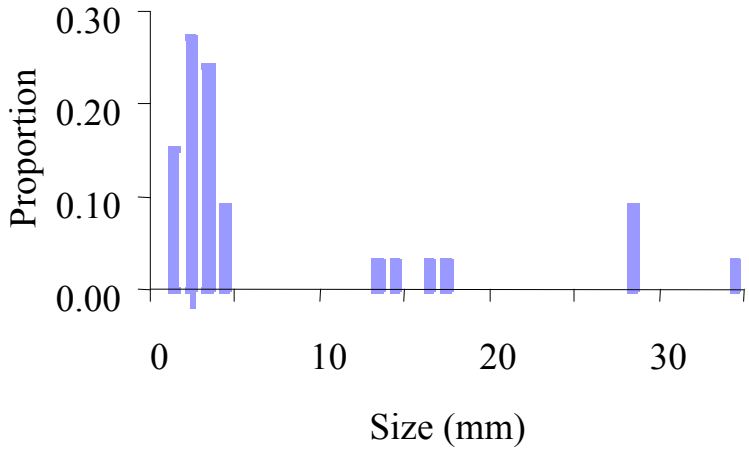


Figure 3.1. Size frequency of wild *Haliotis rufescens* recruits at Wykoff Ledge, San Miguel Island. Abalone were found in random quadrats (n = 13) and were measured in situ to the nearest millimeter.

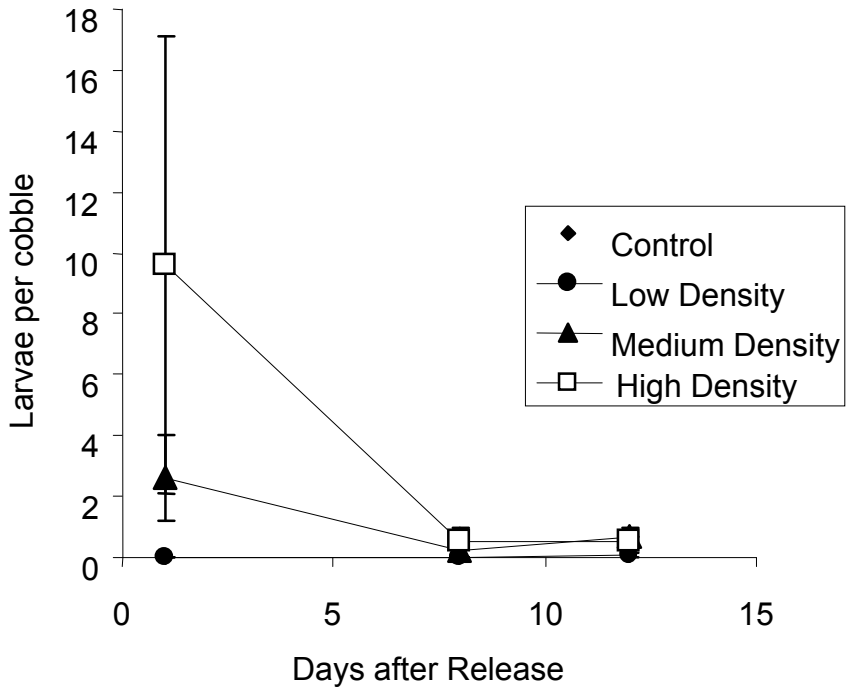


Figure 3.2. Data from the first density release experiment. Larvae in four densities were released into crates of cobble rock (n = 5). Settled larvae were scored at three time intervals. Data are means (+/- 1 SE).

Table 3.3. ANOVA tables for the effect of time and tenting on larval settlement and survivorship. tented (n = 9) and untented (n = 7) crates were sampled at 24 and 48 hours after release of larvae.

Source of Variation	*df*	*MS*	*F*	*P*
Tenting				
Day 1: Tented vs Untented	1	23929141.730	17.739	0.001
Error	14	1348949		
Day 2: Tented vs Untented	1	409302.921	6.236	0.026
Error	14	65639.08		

Source of Variation	*df*	*MS*	*F*	*P*
Day				
Tented: Day 1 vs Day 2	1	21213926.722	17.167	0.001
Error	16	1235739		
Untented: Day1 vs Day 2	1	2828.643	1.048	0.326
Error	12			

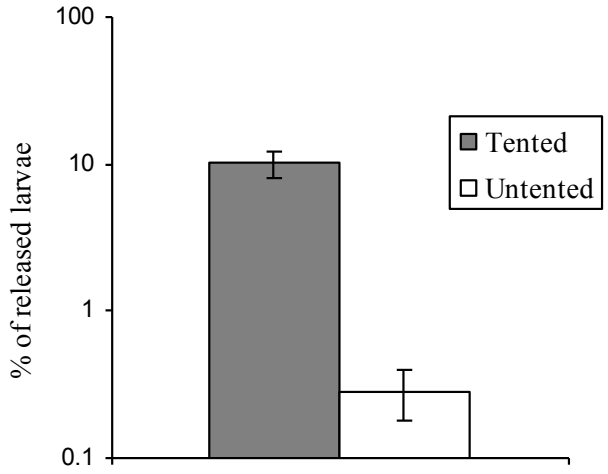


Figure 3.3.a. Fraction of released abalone larvae found settled 24 hours after release, in tented (n = 9) and untented (n = 7) crates. Data are means (+/- 1 SE).

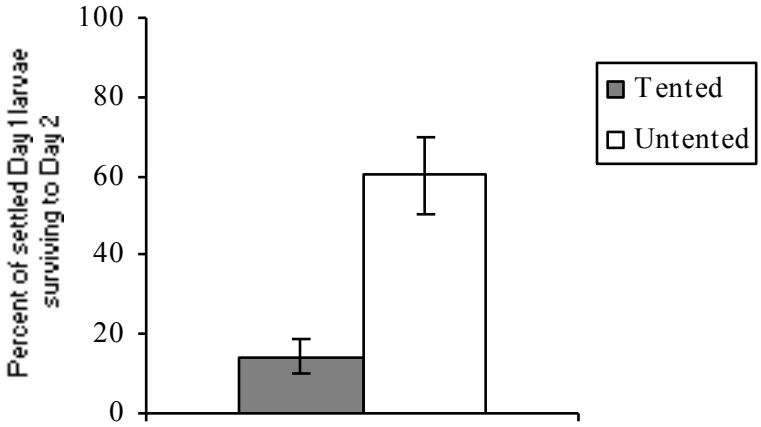


Figure 3.3.b. Mean number of abalone post-settlers at 48 hours shown as a percentage of those settled at 24 hours in tented (n = 9) and untented (n = 7) crates. Data are means (+/- 1 SE).

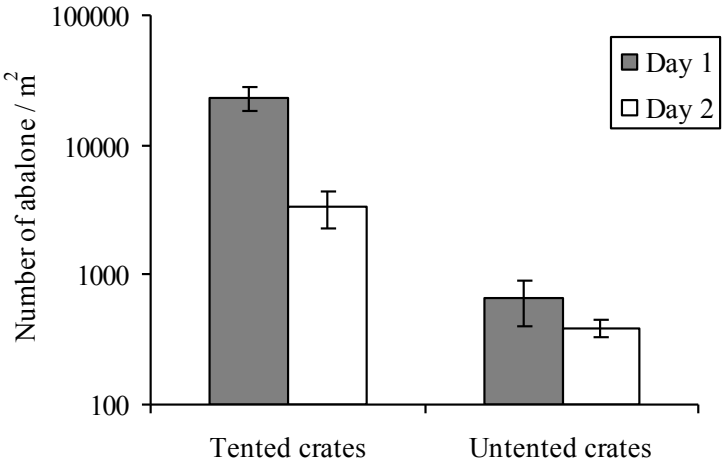


Figure 3.3.c. Density of settled larvae at 24 and 48 hours after larval release, in tented (n = 9) and untented (n = 7) crates. Data are means (+/- 1 SE).

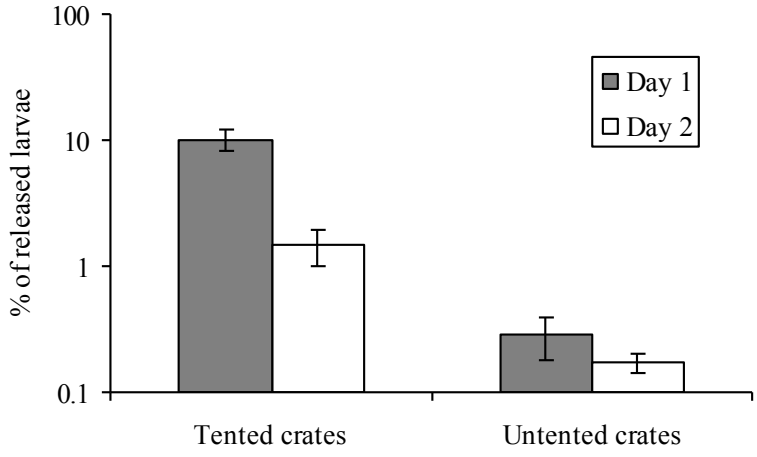


Figure 3.3.d. Settled larvae in tented and untented crates at Day 1 and Day 2 after release of larvae. Values are the mean percent of larvae released (+/- 1 SE).

Table 3.4. Summary data and ANOVA table for the effect of season on settlement and survivorship for each of three sampling periods (1, 7 and 28 days after larval release).

Groups	*# Replicate Crates*	*Mean # Abalone / Crate*	*Variance*
Spring			
1 day	2	131.000	0.000
7 days	4	144.000	4358.667
28 days	9	10.111	189.611
Fall			
1 day	4	3584.750	19653842.250
7 days	5	591.600	864233.800
28 days	6	38.333	4789.067

Source of Variation	*df*	*MS*	*F*	*P*
Season				
1 day	1	15904518.750	1.079	0.358
7 days	1	445212.800	0.898	0.375
28 days	1	2867.378	1.464	0.248

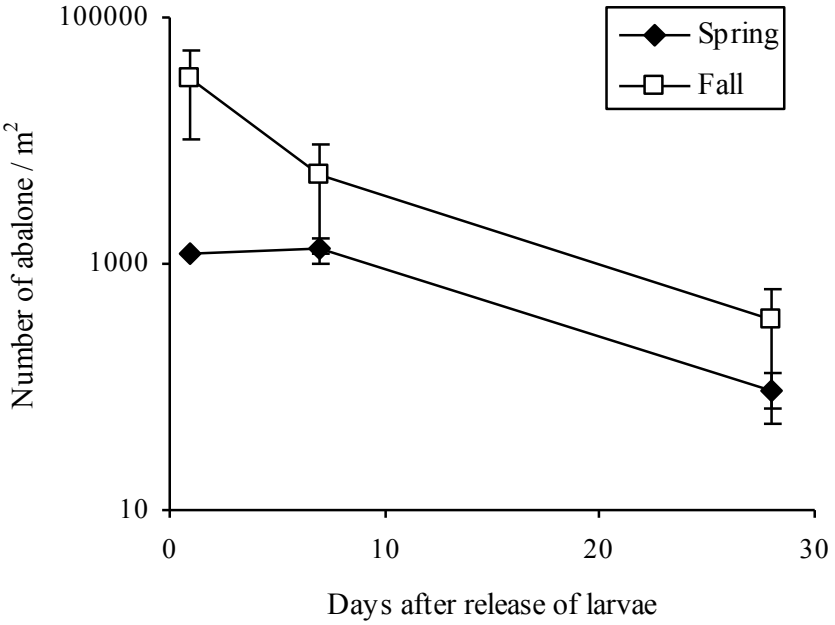


Figure 3.4.a. Abalone density at one, seven and 28 days after release, for both Spring and Fall seasons. Data are means (+/- 1 SE).

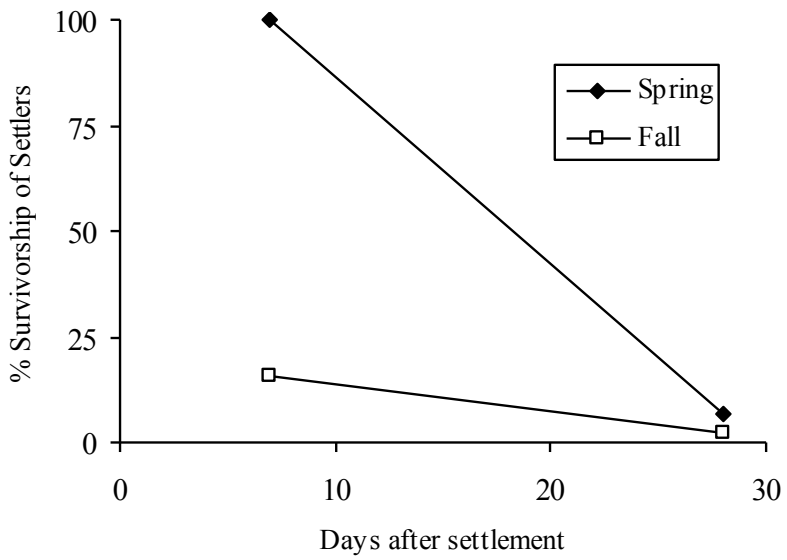


Figure 3.4.b. Abalone survivorship over time in Spring and Fall as a percent of settled larvae at Day 1.

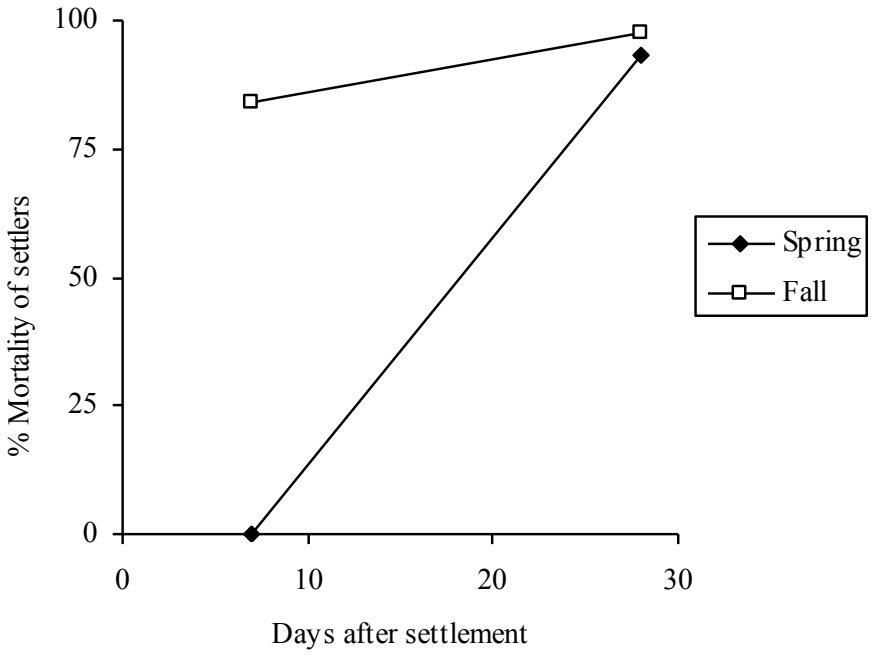


Figure 3.4.c. Abalone mortality over time for two seasons.

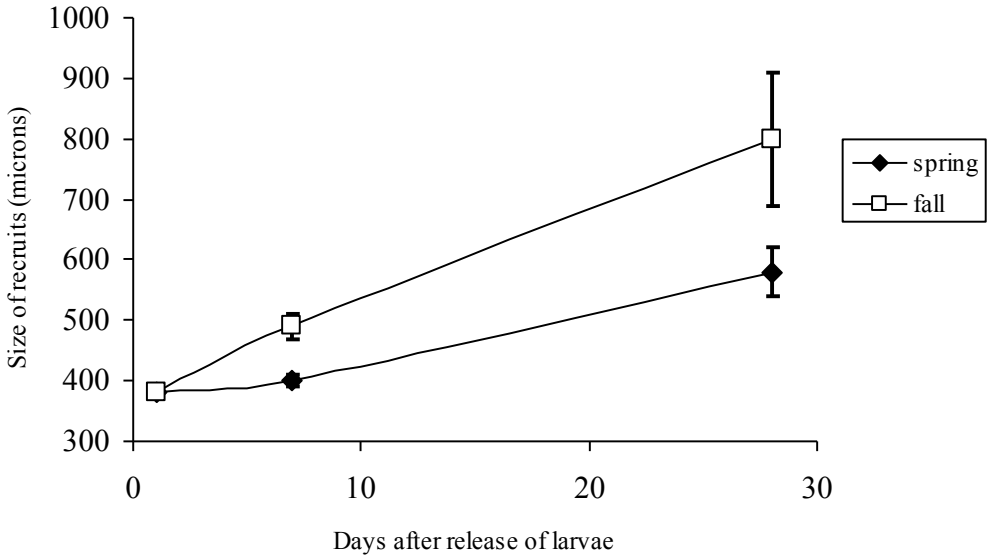


Figure 3.4.d. Abalone recruit growth over time for two different seasons. Data points are means (+/- 1 SE).

Pre-settlement larval shell

1 day post-settlement

2 days post-settlement
Note growth band

Figure 3.4.e. Experimentally released *Haliotis rufescens*, pre- and post-settlement, recovered from field experiment.

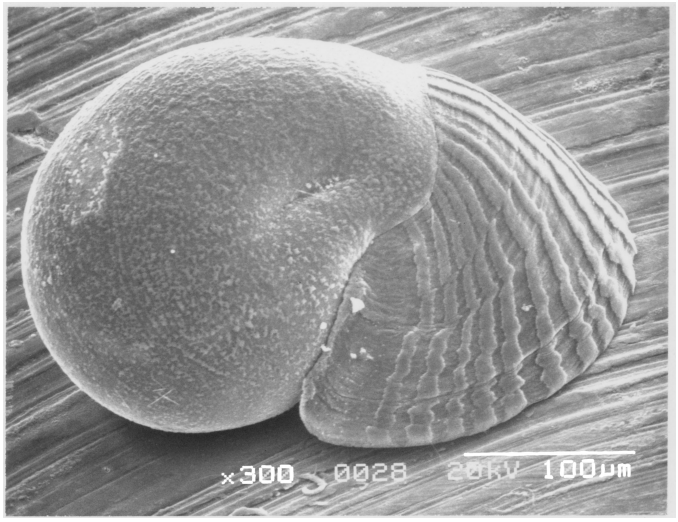


Figure 3.4 f. SEM image of post-settlement larva recovered 7 days after field release.

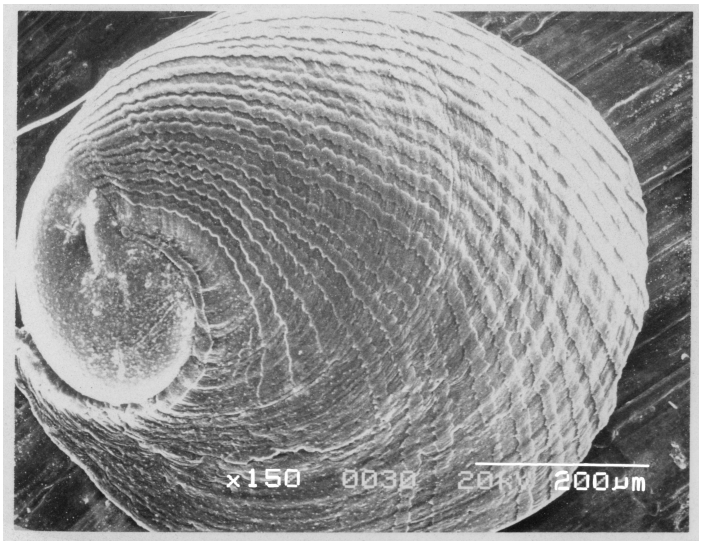
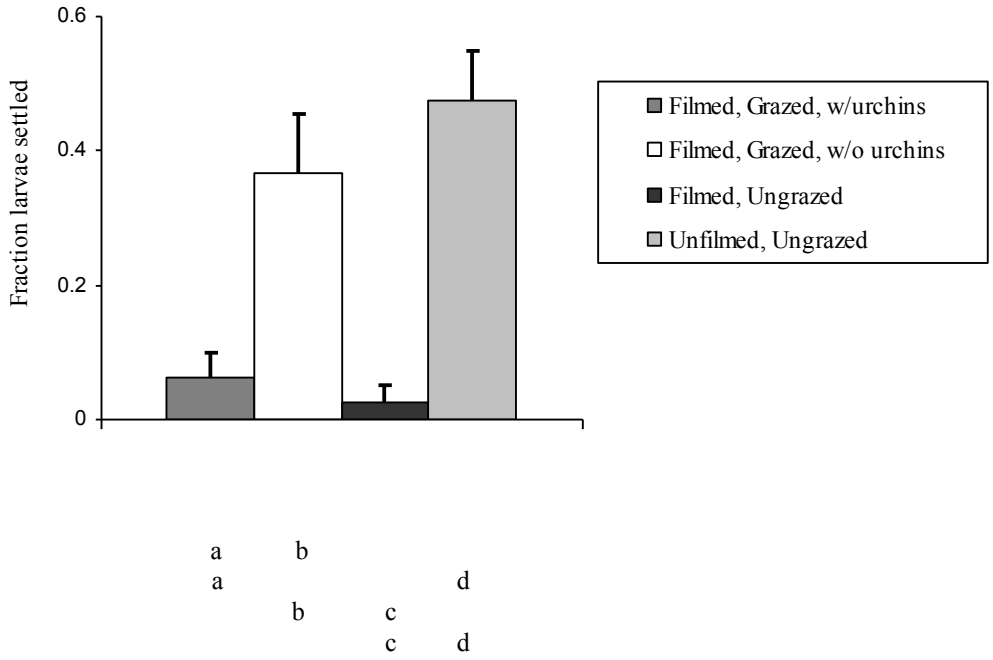


Figure 3.4.g. SEM image of post-settlement larva recovered 28 days after field release.



Significant results are as follows.
ab: P = .0001 F = 7.244
ad: P = .0028 F = 18.087
bc: P = .015 F = 9.504
cd: P = .0014 F = 22.704

ANOVA

Source of Variation	*df*	*MS*	*F*	*P*
Between Groups	1	126.6722	26.42938	0.001337
Error	7	4.792857		

Figure 4.1. Results from the urchin grazing and filming experiments. Data are mean percent larvae settled (+/- 1 SE).

Figure 4.2.a Wild juvenile *Haliotis rufescens* at San Miguel Island. Individual pictured was found under a red sea urchin. Shell length approximately 10 mm.

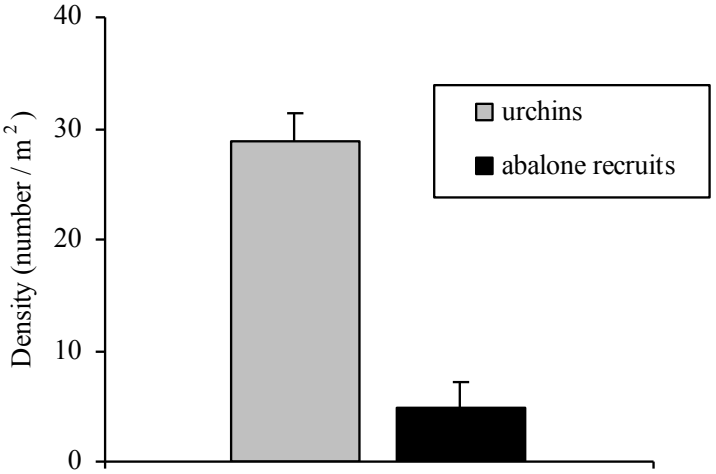


Figure 4.2.b. Density (means + 1 SE) of wild *Haliotis rufescens* recruits and urchins from random quadrats (n = 13) at Wykoff Ledge, San Miguel Island).

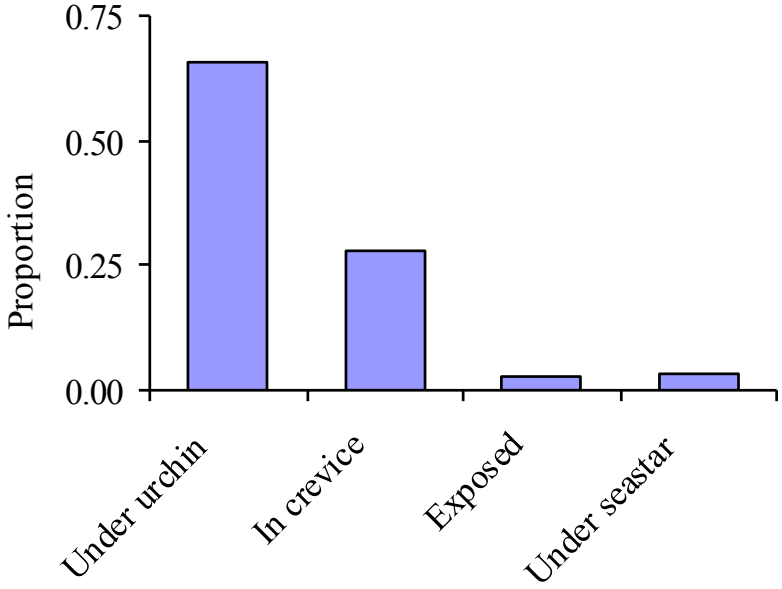


Figure 4.2.c. Proportion of wild post-larval *Haliotis rufescens* found in four different habitats at Nifty Rock, San Miguel Island.

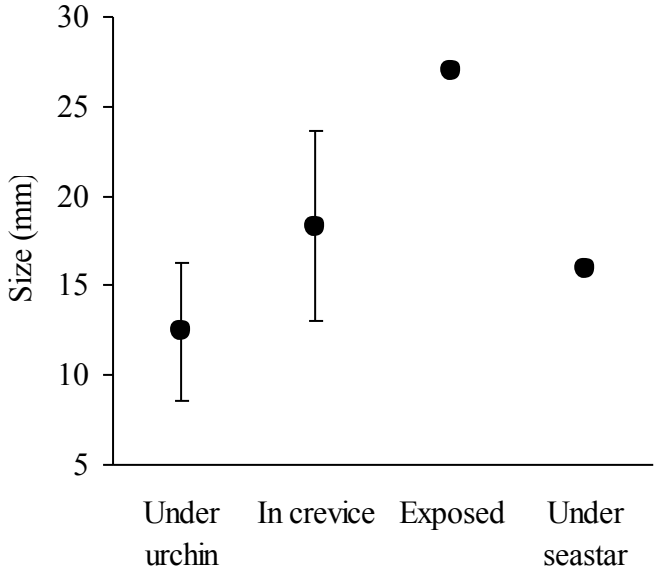


Figure 4.2.d Size of wild juvenile abalone found in four different microhabitats at Nifty Rock, San Miguel Island.

Spring larval release experiment

Days after release of abalone	# abalone recovered	# bivalves recovered	# gastropods recovered
1	131	12	0
1	131	90	12
6	93	61	4
6	119	94	10
6	241	*nd*	*nd*
6	123	263	16
28	12	172	12
28	0	101	9
28	5	114	6
28	5	54	1
28	45	284	32
28	13	250	15
28	2	94	8
28	3	122	11
28	6	3	5

Fall larval release experiment

Days after release of abalone	# abalone recovered	# bivalves recovered	# gastropods recovered
1	2279	17	35
1	104	4	6
1	1874	4	34
1	10082	35	36
7	71	7	12
7	2245	*nd*	*nd*
7	211	*nd*	*nd*
7	105	24	33
7	326	26	9
28	1	10	30
28	14	9	8
28	178	23	2
28	2	5	5
28	6	14	1309
28	29	13	10

Table 4.3. The total number of abalone, bivalve and gastropod recruits recovered from plots for all sampling times, during the Spring and Fall larval release experiments. Plot size = 0.11 m^2.

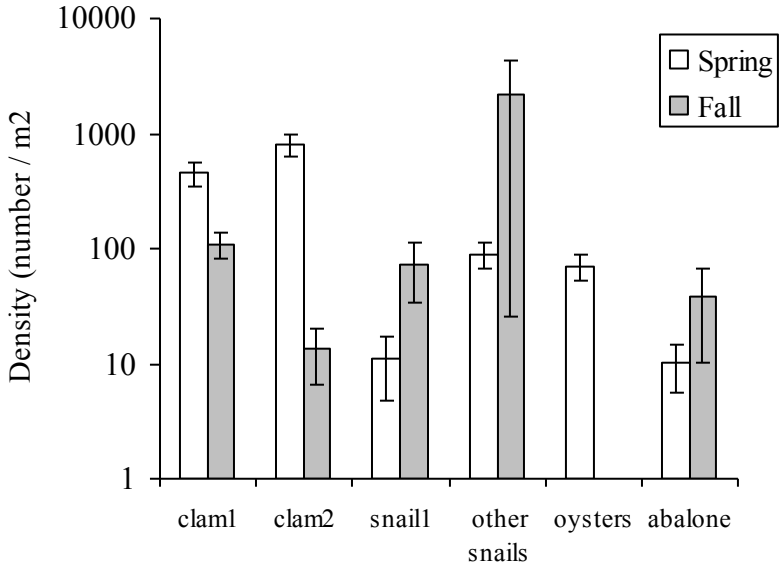


Figure 4.3.1. Five categories of bivalve and gastropod recruits found in abalone plots. Data are mean density (+/- 1SE) of all plots sampled 28 days after release of abalone larvae, for both Spring (n = 9) and Fall (n = 6).

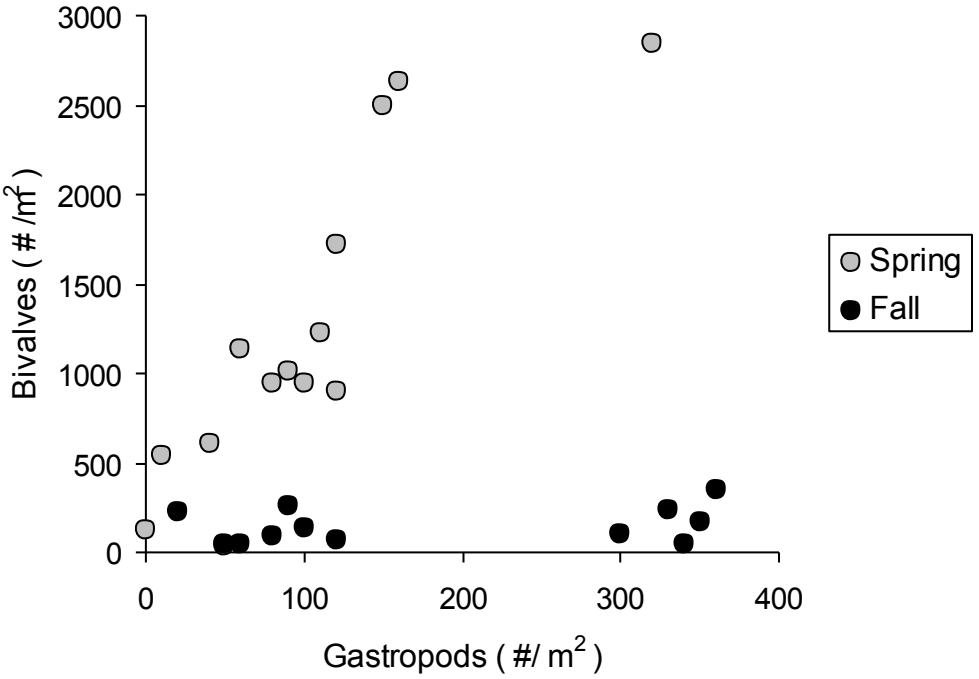


Figure 4.3.2. Densities of bivalve and gastropod recruits for both Spring and Fall larval abalone outplant experiments. Data points represent individual plots for each sampling day.

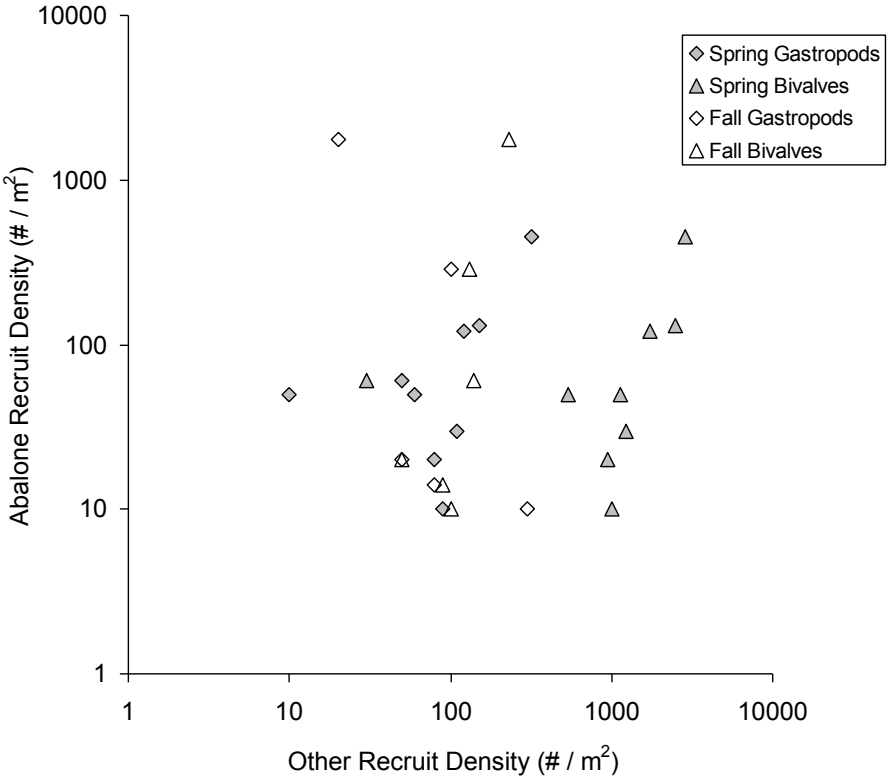


Figure 4.3.3. The relationship between abalone recruits surviving to 28 days and the density of other recruits (bivalves and gastropods) naturally occurring within plots. Data points represent individual plots, for both Spring and Fall experiments.

Figure 5. Losses of experimental units due to entanglement with a lobster trap during a 100-year storm event in 1997.